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(71) **Applicant** *(for all designated States except US)*: **GENENCOR INTERNATIONAL, INC.** [US/US]; 925 Page Mill Road, Palo Alto, CA 94304 (US).

(72) **Inventors; and**
(75) **Inventors/Applicants** *(for US only)*: **DUNN-COLEMAN, Nigel** [US/US]; 142 Johnson Avenue, Los Gatos, CA 95032 (US). **LANGDON, Timothy** [GB/GB]; Troedyrhiw, Lon Tyllwyd, Llanfarian, Aberystwyth SY23 4UJ (GB). **MORRIS, Phillip** [GB/GB]; 6 Trefaenor, Comins-coch, Aberystwyth SY23 3UB, Cerdigion, Wales (GB).

(74) **Agent: BOYD, Victoria, L.**; Genencor International, Inc., 925 Page Mill Road, Palo Alto, CA 94304-1013 (US).

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(54) Title: MANIPULATION OF THE PHENOLIC ACID CONTENT AND DIGESTIBILITY OF PLANT CELL WALLS BY TARGETED EXPRESSION OF GENES ENCODING CELL WALL DEGRADING ENZYMES

(57) **Abstract:** Described herein are methods to enhance the production of more highly fermentable carbohydrates in plannts, especially forage grasses. The invention provides for transgenic plants transformed with expression vectors containing a DNA sequence encoding ferulic acid esterase I from *Aspergillus*, preferably *A. Niger*. The expression vectors may optionally comprise a DNA sequence encoding xylanase from *Trichoderma*, preferably *T. reesei*. Expression of the enzyme(s) is targeted to specific cellular compartments, in specific cellular compartments, in specific tissues and under specific environmental conditions. Uses of this invention include, but are not limited to, forage with improved digestibility for livestock, and enhanced biomass conversion.

MANIPULATION OF THE PHENOLIC ACID CONTENT AND DIGESTIBILITY OF PLANT CELL WALLS BY TARGETED EXPRESSION OF GENES ENCODING CELL WALL DEGRADING ENZYMES

Inventors: **Nigel Dunn-Coleman**
Timothy Langdon
Phillip Morse

CROSS-REFERENCE TO RELATED APPLICATIONS

Pursuant to 35 U.S.C. §119(e), the present application claims benefit of and priority to USSN 60/249,608, entitled "MANIPULATION OF THE PHENOLIC ACID CONTENT AND DIGESTIBILITY OF FORAGE GRASS CELL WALLS BY TARGETED EXPRESSION OF A FERULIC ACID ESTERASE GENE", filed November 17, 2000, by Morris et al.

FIELD OF THE INVENTION

This invention relates to methods to enhance to availability of fermentable carbohydrates.

BACKGROUND OF THE INVENTION

The present crisis in livestock agriculture has prompted a resurgence of interest in grass-fed animals. However, while a high-forage diet may be desirable, it does not currently satisfy the demands of modern animal production. For the animal to make efficient use of the forage it consumes, the energy demands of the micro-organisms in the rumen must be met and synchronized with the availability of plant proteins. Otherwise this lack of synchrony will lead to (a) proteins and other nutrients being poorly utilized in the rumen, (b) loss of nitrogen, in urine and feces and therefore, the environment and (c) the need to feed excessive amounts of protein concentrates as supplements to the ruminant diet.

Cellulose and hemicellulose in grass and maize tissues could meet the

energy requirements of the ruminant or provide new feed-stocks for industrial fermentation to ethanol. This potential is not currently realized because the cell walls are lignified and the cell wall polysaccharides highly cross-linked with phenolic residues and lignin, resulting in low rates of plant cell wall digestion in comparison to rates of protein breakdown in ruminants. This is a particular problem for the most important forages in Europe, the ryegrasses *Lolium perenne* and *L. mutiflorum* as well as one of the major impediments to the wider use of better adapted species, such as *Festuca arundinacea*, as a forage crop. Increasing the digestibility index of grasses has therefore been a major breeding objective for several decades but progress has been slow due to difficulties in fixing natural variation in the synthetic varieties derived from these outbreeding species (Hayward, *et al., TAG* **70**:48 (1985)).

Removing labile phenolics by chemical treatment with alkali is known to increase the biodegradability and nutritional value of low-quality feed such as cereal straw, and is employed commercially for feed upgrading. Reducing phenolic cross-linking of cell wall carbohydrates is therefore a predictable way of improving the rate of digestion and digestibility of ryegrass. However chemical modification may have other disadvantages. Therefore, genetic modification would be a preferable method of changing the cell wall chemistry of highly digestible varieties. Many in the field are pursuing this approach. An alternative, however, is to use genetic modification to reduce the levels of phenolic acids in the cell walls available for crosslinking either by directly disrupting ester bonds linking phenolics and lignins to cell wall polysaccharides or by preventing excessive ferulation of cell wall carbohydrates prior to their incorporation into the cell wall.

This invention meets this and other needs by using targeted or inducible expression of cell wall degrading enzymes in plants.

SUMMARY OF THE INVENTION

Provided herein are methods for enhancing the availability of fermentable carbohydrates. In one aspect, there is provided an expression cassette comprising a DNA sequence encoding at least one cell wall degrading enzyme. The DNA sequence encoding at least one cell wall degrading enzyme may be operatively linked to a promoter sequence. The promoter may be constitutive or inducible. The expression cassette may further comprise a targeting sequence.

In one embodiment, the cell wall degrading enzyme is selected from the group consisting of ferulic acid esterase, xylanase, xylosidase, cellulase, endoglucanase, and cellbiohydrolase. In a preferred embodiment cell wall degrading enzyme is derived from a fungal source. In a more preferred embodiment, the fungal ferulic acid esterase is an *Aspergillus* ferulic acid esterase, preferably *A. niger*. In another embodiment the xylanase is derived from *Trichoderma*, preferably *T. reesei*.

In another aspect of the invention, there is provided a plant transformed with the expression cassette comprising a DNA sequence encoding at least one cell degrading enzyme. The plant may be selected from the group consisting of Festuca, Lolium, Avena and Zea. In a preferred embodiment the plant is a forage grass. In another embodiment, the plant is maize.

Further provided herein is a method of controlling the level of phenolic acids in plant cell walls of a transgenic plant. The method, in one embodiment, comprises introducing to a plant cell an expression cassette comprising a DNA sequence encoding at least one cell wall degrading enzyme, preferably a ferulic acid esterase.

Other objects, features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only, since various changes and modifications within the scope and spirit of the invention will become apparent to one skilled in the art from this detailed description.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 illustrates a restriction map of a DNA fragment containing the gene encoding the 38kd ferulic acid esterase.

Figures 2 A-E illustrate the complete DNA (SEQ. ID NO:__), with highlighting to point out the signal sequence, intron and various restriction endonuclease sites, and amino acid sequence (SEQ. ID. NO:__) corresponding to the gene encoding the 38 kD ferulic acid esterase isolated from *Aspergillus niger.*

Figure 3 illustrates the DNA sequence of the gene encoding the 38 kD esterase (SEQ. ID. NO:__).

Figure 4 illustrates the construction of the intronless ferulic acid esterase isolated from *Aspergillus niger.*

Figure 5 illustrates that the overlapping of PCR products made with primers FAE-I5 FAE-I3 creates two possible uninterrupted reading frames – the top in the figure below is functional (highlighted serine is at active site), the bottom is inactivated.

Figure 6 illustrates the possible vector constructions useful in the present invention. Various combinations are possible. Although and FAE gene is depicted another cell wall degrading enzyme may be used alone (i.e., instead of) or in conjunction with the FAE gene. Amp = ampicillin resistance gene.

Figure 7 illustrates pCOR105.

Figure 8 illustrates a generic ALE-TER vector.

Figure 9 illustrates the KDEL-COOH ER retention sequences.

Figure 10 illustrates the FAE-LINKER-FRAMESHIFT structure and sequence.

Figure 11 illustrates plant transformation cassettes.

Figure 12 is a table of the vectors used herein.

Figure 13 depicts the barley aleurain vacuolar and apoplast signal sequence.

Figure 14 illustrates the rat sialyl transferase structure and sequence.

Figure 15 illustrates the potato protease inhibitor II (PPI) motif structure and sequence.

Figure 16 illustrates the targeted expression of gfp to different cell compartment. Also shown are schematics of the vectors used.

Figure 17 illustrates the FAE activity in transgenic *Festuca arundinacea* leaves of different ages under ER and APO targeting sequences.

Figure 18 illustrates the FAE activity in transgenic *Festuca arundinacea* leaves of different ages under Vac targeting sequence.

Figure 19 illustrates the FAE activity in transgenic *Lolium mutflorum* leaves of different ages.

Figure 20 illustrates the FAE activity in transgenic *Lolium mutflorum* leaves under Vac, ER and APO targeting sequences.

Figure 21 illustrates the levels of esterified monomeric and dimeric hydroxycinnamic acids in *Festuca arundinacea* plants expressing FAE under Vac targeting sequence.

Figure 22 illustrates the levels of esterified monomeric and dimeric hydroxycinnamic acids in *Festuca arundinacea* plants expressing FAE under APO and ER targeting sequence.

Figure 23 illustrates the *in vitro* dry matter digestibility of leaf tissue of mature *Festuca arundinacea* plants expressing FAE under an actin promoter.

Figure 24 illustrates the *in vitro* dry matter digestibility of leaf tissue of mature *Lolium mutflorum* plants expressing FAE under an actin promoter.

Figure 25 illustrates the rate of fermentation and cumulative gas production in *Festuca arundinacea* cells.

Figure 26 illustrates the in vitro fermentation of *Festuca arundinacea* cell walls from cell cultures expressing recombinant FAE1.

Figure 27 illustrates the Time to maximum rate digestion for *Festuca arundinacea* cells.

Figure 28 illustrates the total gas production in *Festuca arundinacea* cells.

Figure 29 illustrates the kinetics of FAE activity by ferulic acid release from cell wall under self digestion in *Festuca arundinacea* and stimulation by xylanase.

Figure 30 illustrates the beta-glucoronidase activity under the Lolium See1 senescence promoter in leaves of transgenic plants of *Lolium mutflorum*.

Figure 31 illustrates the release of monomeric and dimeric HCAs on self digestion of leaves of vacuolar targeted FAE expressing plants.

Figure 32 is a schematic of the pTP10-1 vector. Also shown is the 5338 bp nucleotide sequence of the vector.

Figure 33 is a schematic of the pUA4-4 vector. Also shown is the 5345 bp nucleotide sequence of the vector.

Figure 34 is a schematic of the pTU4 vector. Also shown is the 5337 bp nucleotide sequence of the vector.

Figure 35 is a schematic of the pTT5.14 vector. Also shown is the 5395 bp nucleotide sequence of the vector.

Figure 36 is a schematic of the pTP8-5 vector. Also shown is the 5337 bp nucleotide sequence of the vector.

Figure 37 is a schematic of the pTP5-1 vector. Also shown is the 5277 bp nucleotide sequence of the vector.

Figure 38 is a schematic of the pTP4a2 vector. Also shown is the 5327 bp nucleotide sequence of the vector.

Figure 39 is a schematic of the pTP3-1 vector. Also shown is the 5338 bp nucleotide sequence of the vector.

Figure 40 is a schematic of the pTU5 vector. Also shown is the 5337 bp nucleotide sequence of the vector.

Figure 41 is a schematic of the pGT6 vector. Also shown is the 4773 bp nucleotide sequence of the vector.

Figure 42 is a schematic of the pJQ5 vector. Also shown is the 5034 bp nucleotide sequence of the vector.

Figure 43 is a schematic of the pJO6.1 vector. Also shown is the 4950 bp nucleotide sequence of the vector.

Figure 44 is a schematic of the pJQ4 vector. Also shown is the 4974 bp nucleotide sequence of the vector.

Figure 45 is a schematic of the pPQ10.1 vector. Also shown is the 5164 bp nucleotide sequence of the vector.

Figure 46 is a schematic of the pJQ3 vector. Also shown is the 4965 bp nucleotide sequence of the vector.

Figure 47 is a schematic of the pUG4 vector. Also shown is the 5295 bp nucleotide sequence of the vector.

Figure 48 is a schematic of the pUB8.11 vector. Also shown is the 5001 bp nucleotide sequence of the vector.

Figure 49 is a schematic of the pTP11-1 vector. Also shown is the 5387 bp nucleotide sequence of the vector.

Figure 50 illustrates the actin promoter and its corresponding nucleotide sequence.

Figure 51 illustrates the Aleurain-NPIR delete structure. The corresponding nucleotide sequences are also shown.

Figure 52 illustrates the SEE1 (senescence enhanced) promoter sequence.

Figure 53 illustrates the SEE1 (senescence enhanced) promoter sequence plus the vacuolar aleurain signal/NPIR sequence.

DETAILED DESCRIPTION OF THE INVENTION

The invention will now be described in detail by way of reference only using the following definitions and examples. All patents and publications, including all sequences disclosed within such patents and publications, referred to herein are expressly incorporated by reference.

Unless defined otherwise herein, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Singleton, *et al.*, DICTIONARY OF MICROBIOLOGY AND MOLECULAR BIOLOGY, 2D ED., John Wiley and Sons, New York (1994), and Hale & Marham, THE HARPER COLLINS DICTIONARY OF BIOLOGY, Harper Perennial, NY (1991) provide one of skill with a general dictionary of many of the terms used in this invention. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are described. Numeric ranges are inclusive of the numbers defining the range. Unless otherwise indicated, nucleic acids are written left to right in 5' to 3' orientation; amino acid sequences are written left to right in amino to carboxy

orientation, respectively. Practitioners are particularly directed to Sambrook *et al.*, 1989, and Ausubel FM *et al.*, 1993, for definitions and terms of the art. It is to be understood that this invention is not limited to the particular methodology, protocols, and reagents described, as these may vary.

The headings provided herein are not limitations of the various aspects or embodiments of the invention which can be had by reference to the specification as a whole. Accordingly, the terms defined immediately below are more fully defined by reference to the specification as a whole.

Definitions

It should be noted that, as used in this specification and the appended claims, the singular forms "a," "an, "and "the" include plural referents unless the content clearly dictates otherwise. Thus, for example, reference to a composition containing "a compound" includes a mixture of two or more compounds. It should also be noted that the term "or" is generally employed in its sense including "and/or" unless the content clearly dictates otherwise.

"Conservatively modified variants" applies to both amino acid sequences and polynucleotides. With respect to particular polynucleotides, conservatively modified variants refers to those polynucleotides that encode identical or essentially identical amino acid sequences, or where the polynucleotide does not encode an amino acid sequence, to essentially identical sequences. Because of the degeneracy of the genetic code, a large number of functionally identical polynucleotides encode any given protein. For instance, the codons GCA, GCC, GCG and GCU all encode the amino acid alanine. Thus, at every position where an alanine is specified by a codon, the codon can be altered to any of the corresponding codons described without altering the encoded polypeptide. Such nucleic acid variations are "silent variations," which are one species of conservatively modified variations. Every polynucleotide herein which encodes a polypeptide also describes every possible silent variation of the nucleic acid. One of skill will recognize that each codon in a polynucleotide (except AUG, which is ordinarily the only codon for methionine) can be modified to yield a functionally identical molecule. Accordingly, each silent variation of a polynucleotide which encodes a polypeptide is implicit in each described

sequence. For purposes of protein expression, there are "sub-optimal codons." These are codons that are not preferred by a particular genus or species. Altering these "sub-optimal codons" to "preferred codons" is a silent mutation in that the amino acid encoded by the codons is the same but one codon is preferentially expressed by the particular genus, *e.g., Triticum spp.*

As to amino acid sequences, one of skill will recognize that individual substitutions, deletions or additions to a polynucleotide, peptide, polypeptide, or protein sequence which alters, adds or deletes a single amino acid or a small percentage of amino acids in the encoded sequence is a "conservatively modified variant" where the alteration results in the substitution of an amino acid with a chemically similar amino acid. Conservative substitution tables providing functionally similar amino acids are well known in the art.

The following six groups each contain amino acids that are conservative substitutions for one another:

1) Alanine (A), Serine (S), Threonine (T);
2) Aspartic acid (D), Glutamic acid (E);
3) Asparagine (N), Glutamine (Q);
4) Arginine (R), Lysine (K);
5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V); and
6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W).

(*see, e.g.*, Creighton, *Proteins* (1984)).

"Pyroglutamic acid" is the cyclized internal amide of L-glutamic acid

The phrase "controlling the level of phenolic acids" refers to the manipulation of phenolic acid expression in plants, particularly plant cell walls. The manipulation can be either positive; *e.g.,* increasing the levels of phenolic acids; negative, *e.g.,* decreasing the level of phenolic acids; or neutral, *e.g.,* changing the relative amounts of specific phenolic acids in the cell walls but keeping the total amount relatively the same. The timing of manipulation can be during plant growth or after plant growth, *e.g.,* after a plant has been cut or pulled from the ground or ingested. "Plant cell walls" refers to the cell walls of any cell of the plant.

The term "derived" means that a polynucleotide or protein is related to

another polynucleotide or protein. The relations can be one of homology, *e.g.*, nucleotides and proteins from certain species are homologous to similar polynucleotides and proteins of other species; analogy, *e.g.*, proteins perform the same function and therefore are related to each other regardless of organism of origin. The relationship can be a man-made one, *e.g.*, a protein (and a polynucleotide) can be derived from another protein by mutation; or chemical manipulation (peptidomimetics). Furthermore, a protein or a polynucleotide can be derived from an organism if, in the natural state, the protein or polynucleotide is found in one organism but recombinantly produced in another.

The term "exogenous polynucleotide" refers to a polynucleotide which is introduced into the plant by any means other than a sexual cross or sexual reproduction. Examples of means by which this can be accomplished are described below, and include *Agrobacterium*-mediated transformation, biolistic methods, electroporation, *in planta* techniques, and the like. Such a plant containing the exogenous polynucleotide is referred to here as an R_1 generation transgenic plant. Transgenic plants which arise from sexual cross or by selfing are progeny of such a plant.

The term "isolated polynucleotide molecule" or "isolated protein" refers to a polynucleotide or protein which is essentially free of other cellular components with which it is associated in the natural state. It is preferably in a homogeneous state although it can be in either a dry or aqueous solution. Purity and homogeneity are typically determined using analytical chemistry techniques such as polyacrylamide gel electrophoresis or high performance liquid chromatography. A protein which is the predominant species present in a preparation is substantially purified. In particular, an isolated *FAE1* gene is separated from open reading frames which flank the gene and encode a protein other than FAE1.

A "*FAE1* encoding polynucleotide" is a nucleic acid sequence comprising (or consisting of) a coding region of an FAE 1 gene or which encodes a FAE1 polypeptide. *FAE1* polynucleotides can also be identified by their ability to hybridize under low stringency conditions (see below) to nucleic acid probes having a sequence of 8 to 300 bases, preferably a sequence of 80 to 100 bases

in the sequence disclosed in WO 98/14594.

The term "nucleic acid encoding," "nucleic acid sequence encoding" or "polynucleotide encoding" refers to a polynucleotide which directs the expression of a specific protein or peptide. The polynucleotides include both the DNA strand sequence that is transcribed into RNA and the RNA sequence that is translated into protein. The polynucleotides include both full length polynucleotides as well as shorter sequences derived from the full length sequences. It is understood that a particular polynucleotide includes the degenerate codons of the native sequence or sequences which may be introduced to provide codon preference in a specific host cell. The polynucleotide includes both the sense and antisense strands as either individual single strands or in the duplex form.

The term "operably linked" refers to functional linkage between a promoter and a second sequence, wherein the promoter sequence initiates transcription of RNA corresponding to the second sequence.

The term "plasmid" refers to a circular double stranded DNA molecule which comprises the coding sequence of interest, regulatory elements, a selection marker and optionally an amplification marker. A plasmid can transform prokaryotic cells or transfect eukaryotic cells. An "expression cassette" means a portion of a plasmid (or the entire plasmid) containing the regulatory elements desired for transcription, translation and/or expression and the coding region of a polynucleotide. A plasmid may contain one or more expression cassettes. If multiple expression cassettes are introduced into a plant, they can be introduced simultaneously or at different times. If simultaneous introduction is desired, the expression cassettes can be on one plasmid or more. Typically, an expression cassette comprises a promoter, poly A+ tail, and signal sequences that target the expressed polypeptide to a specific region of a cell or to be secreted, if desired. Examples of signal sequences that "target expression" of ferulic acid esterase include sequences located upstream of the FAE coding sequence. The polynucleotide that encodes the signal sequence is found preferably within the 100 nucleotides "upstream" (in the 5' direction) from the initiation codon (AUG). More preferably, the polynucleotide that encodes the signal sequence is found within the 50 nucleotides upstream from the initiation

codon. Many different cellular organelles are targeted by the signal sequences used in this invention. The organelles include, but are not limited to, vacuoles, Golgi apparati, endoplasmic reticula, and apoplasts. In addition to upstream signal sequences, the expression cassette of this invention may include a polynucleotide that encodes a signal sequence at the 3' end. These signal sequences include, but are not limted to stop codons and the KDEL sequence. In addition to KDEL, other similar sequences are contemplated by this invention, including but not limited to RDEL. In addition to a KDEL sequence, a signal sequence can include a linker to a KDEL sequence. A linker is an extension of the reading frame of the encoding polynucleotide to the signal sequence. Preferably, the polynucleotide encoding the signal sequence is directly downstream from the coding sequence, more preferably less than 100 base pairs from the stop codon, more preferably less than 20 base pairs from the stop codon.

The term "polynucleotide," "polynucleotide" or "nucleic acid sequence" refers to deoxyribonucleotides or ribonucleotides and polymers thereof in either single- or double-stranded form. Unless specifically limited, the term encompasses polynucleotides containing known analogues of natural nucleotides which have similar binding properties as the reference polynucleotide and are metabolized in a manner similar to naturally occurring nucleotides. Unless otherwise indicated, a particular *FAE1* polynucleotide of this invention also implicitly encompasses conservatively modified variants thereof (*e.g.* degenerate codon substitutions) and complementary sequences and as well as the sequence explicitly indicated. Specifically, degenerate codon substitutions may be achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (Batzer *et al.*, *Nucleic Acid Res.* **19**:5081 (1991); Ohtsuka *et al.*, *J. Biol. Chem.* **260**:2605-2608 (1985); and Cassol *et al.*, 1992; Rossolini *et al.*, *Mol. Cell. Probes* **8**:91-98 (1994)). The term polynucleotide is used interchangeably with gene, cDNA, and mRNA encoded by a gene.

The term "polypeptide," "peptide," and "protein" are used interchangeably and refer to amino acids connected by peptide bonds. Polypeptides can be

entire proteins or portions thereof. For Example. a FAE1 polypeptide may refer to the entire FAE1 protein or fragments of the FAE1 protein. A "ferulic acid esterase with an altered glycosylation site" refers to a FAE protein wherein a mutation has changed the glycosylation pattern of the protein. Mutations that effect such changes are well known in the art and include, but are not limited to, amino acid substitutions, and mutations in the proteins of the Golgi apparatus and endoplasmic reticulum that effect glycosylation of proteins.

The term "promoter" refers to a polynucleotide that directs expression of a coding sequence. A promoter can be constitutive, *i.e.,* relatively independent of the stage of differentiation of the cell in which it is contained or it can be inducible, *i.e.,* induced be specific environmental factors, such as the length of the day, the temperature, *etc.* or a promoter can be tissue-specific, *i.e.,* directing the expression of the coding sequence in cells of a certain tissue type. A "senescence" promoter is an inducible promoter that causes transcription to be initiated upon a certain event relating to age of the organism. A "heat shock promoter" is an inducible promoter that causes transcription to be initiated upon a change in temperature. An example of a heat shock protein promoter is the Soybean Gmhsp promoter. In addition to these inducible promoters, one of skill will realize that other inducible promoters can be used. For example, a wound induced promoter, like LAP. See, US Patent No. 5,962,670.

The term "purified" denotes that a polynucleotide or protein gives rise to essentially one band in an electrophoretic gel. Particularly, it means that the polynucleotide or protein is at least 85% pure, more preferably at least 95% pure, and most preferably at least 99% pure.

The term "specifically hybridizes" refers to a nucleic acid probe that hybridizes, duplexes or binds to a particular target DNA or RNA sequence when the target sequences are present in a preparation of total cellular DNA or RNA. "Complementary" or "target" nucleic acid sequences refer to those nucleic acid sequences which selectively hybridize to a nucleic acid probe. Proper annealing conditions depend, for example, upon a probe's length, base composition, and the number of mismatches and their position on the probe, and must often be determined empirically. For discussions of nucleic acid probe design and

annealing conditions, see, for example, Sambrook *et al.*, MOLECULAR CLONING: A LABORATORY MANUAL (2ND ED.), Vols. 1-3, Cold Spring Harbor Laboratory, (1989) ("Sambrook") or CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, F. Ausubel *et al.*, ed. Greene Publishing and Wiley-Interscience, New York (1987) ("Ausubel").

The term "stringent conditions" in the context of polynucleotide hybridization experiments such as Southern and northern hybridizations refers to sequence dependent, binding and washing environments. An extensive guide to the hybridization of polynucleotides is found in Tijssen (1993) LABORATORY TECHNIQUES IN BIOCHEMISTRY AND MOLECULAR BIOLOGY--HYBRIDIZATION WITH NUCLEIC ACID PROBES part I chapter 2 "overview of principles of hybridization and the strategy of nucleic acid probe assays", Elsevier, New York. Generally, highly stringent hybridization and wash conditions are selected to be about 5°C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH. The T_m is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. Very stringent conditions are selected to be equal to the T_m for a particular probe. An example of stringent hybridization conditions for hybridization of complementary polynucleotides which have more than 100 complementary residues on a filter in a Southern or northern blot is 50% formalin with 1 mg of heparin at between 40 and 50°C, preferably 42°C, with the hybridization being carried out overnight. An example of highly stringent wash conditions is 0.15M NaCl at from 70 to 80°C with 72°C being preferable for about 15 minutes. An example of stringent wash conditions is a 0.2x SSC wash at about 60 to 70°C, preferably 65°C for 15 minutes (*see*, Sambrook, *supra* for a description of SSC buffer). Often, a high stringency wash is preceded by a low stringency wash to remove background probe signal. An example medium stringency wash for a duplex of, *e.g.*, more than 100 nucleotides, is 1x SSC at 40 to 50°C, preferably 45°C for 15 minutes. An example low stringency wash for a duplex of, *e.g.*, more than 100 nucleotides, is 4-6x SSC at 35 to 45°C, with 40°C being preferable, for 15 minutes. In general, a signal to noise ratio of 2x (or higher) than that observed for an unrelated probe in the particular hybridization assay indicates detection of a specific hybridization. Polynucleotides which do

not hybridize to each other under stringent conditions are still substantially identical if the polypeptides which they encode are substantially identical. This occurs, *e.g.*, when a copy of a polynucleotide is created using the maximum codon degeneracy permitted by the genetic code.

The term "transgenic plant" refers to a plant into which exogenous polynucleotides have been introduced and their progeny. Typically, cells of a plant are transformed with the exogenous polynucleotide and a transgenic plant is regenerated from the transformed cells. The regenerated plant is then bred to produce a strain of transgenic plants.

"Xylanase" (EC 3.2.1.8) refers to a well described class of gylcosyl hydrolases that hydrolize xylan. Commercial applications of xylanase include the degradation and bleaching of wood pulp for paper making. Xylanase can also be added to animal feed to improve the digestibility of plant matter. Typically, commercial xylanase is derived from fungi. A preferred xylanase is derived from *Trichoderma*.

Preferred Embodiments

Plant cell walls contain a range of alkali-labile ester-linked phenolic acids. In particular, grass cell walls are characterized by the presence of large amounts of esterified ferulic and p-coumaric acids (mainly in their E configurations), linked to arabinoxylans at the C5 of arabinose. These are released as ferulated oligosaccharides (FAX and PAX) by cellulase treatment but *in vivo* provide a substrate for peroxidase-catalyzed cross-linking of cell wall polysaccharides and lignin. The high levels of these phenolic acids and their dimers have a dramatic influence on the mechanical properties, digestibility and rates of digestion of grasses by ruminants.

Previous work has shown that ferulic acid is the predominant p-hydroxycinnamic acid esterified to grass polysaccharide but until recently the only ferulic acid dehydrodimer to have been isolated was 5,5'-diferulic acid. Recently new dehydrodiferulate dimers and cyclobutane-type dimer mixtures have been isolated from plant cell walls (Waldron, *et al., Phytochemical Analysis* 7:305 (1996)). As can be seen in Figure 1, these mixtures are present in large amounts in grass cells. Ether linked ferulic acid-coniferyl alcohol dimers, have also been isolated from cell walls (Jacquet, *et al., Polyphenol Comm. Bordeaux*

pp451 (1996)) establishing for the first time that ferulate esters are oxidatively co-polymerized with lignin precursors which may anchor lignins to cell wall polysaccharides. The yield of these dimers in grass cells indicates that phenolic dehydrodimer cross-linking of cell wall polysaccharides is much more extensive than was previously thought.

An enzyme system has been reported from parsley endomembranes that catalyses the ferulation of endogenous polysaccharide acceptors from feruloyl CoA, pointing to the ER/golgi as the site of polysaccharide esterification and the CoA ester as the physiological co-substrate (Meyer, *et al., FEBS Lett.* **290**:209 (1991)). Further evidence for this has been found in water-soluble extracellular polysaccharides excreted in large amounts into the medium by grass cell cultures. This material is highly esterified with ferulic and p-coumaric acid at levels similar to the cell walls of the cultured cells.

Feruloyl esterase activity has been detected in several fungal species including, anaerobic gut fungi, yeasts, actinomycetes, and a few fiber-degrading ruminal bacteria, which enables them to de-esterify arabinoxylans and pectins.

Two ferulic acid esterases (FAE), distinguished on the basis of molecular weight and substrate specificity, have been isolated from *Aspergillus niger* and have been shown to quantitatively hydrolyze ferulic acid and release dehydrodiferulate dimers from plant cell walls. Furthermore, FAE has been observed to act synergistically with xylanase to release ferulic acid from plant cell walls at a higher rate. Recently, a ferulic acid esterase (FAE) gene has been cloned from *Aspergillus niger* (Michelson, *et.al.* European Patent Application No. 9510370.1). The inventors have found the recombinant enzyme releases ferulic acid and diferulate dimers from grass cell walls in a concentration dependent manner and that this enzyme is stable at 30°C pH 5.0 in the presence of substrate and has a half life of 61 h at 30°C in the presence of vacuolar extracts (pH 4.6) of grass cells. This gene was, therefore, a candidate for targeted and indicible expression of FAE in grasses (*e.g., Lolium multiflorum*).

The present invention provides for methods of changing the cell wall structure of transgenic plants and therefore, making them more digestible. The method comprises introducing a ferulic acid esterase coding sequence into the

cells of a plant. Operably linked to the coding seqeunce is a promoter that can be either constitutive or inducible and signal sequences that serve to target expression of the coding sequence in the desired organelle in the desired cell of the plant. The signal sequences can be either or both N terminal or C terminal sequences.

Optionally, a second and/or third coding sequence is introduced into the plant. It is preferred that a fungal xylanase coding sequence be coexpressed with the FAE coding sequence. .

This invention also provides for transgenic plants which contain FAE1 coding sequences, leading to more digestible grasses.

Generally, the nomenclature and the laboratory procedures in recombinant DNA technology described below are those well known and commonly employed in the art. Standard techniques are used for cloning, DNA and RNA isolation, amplification and purification. Generally enzymatic reactions involving DNA ligase, DNA polymerase, restriction endonucleases and the like are performed according to the manufacturer's specifications. Basic texts disclosing the general methods of use in this invention include Sambrook, *et al.*, MOLECULAR CLONING, A LABORATORY MANUAL, 2ND ED. (1989); Kriegler, GENE TRANSFER AND EXPRESSION: A LABORATORY MANUAL (1990); and Ausubel *et ál.*, (eds.), CURRENT PROTOCOLS IN MOLECULAR BIOLOGY (1994)).

A. Isolation of Polynucleotides

The isolation of the polynucleotides, *e.g.*, FAE1 and xylanase of the invention may be accomplished by a number of techniques. See, for example, copending US application 08/952,445 which describes the isolation of a FAE from *Aspergillus niger*, and copending US application 09/658,772 which describes the isolation of a xylanase from T. reesei.

For instance, oligonucleotide probes based on the sequences cited here can be used to identify the desired gene in a cDNA or genomic DNA library from a desired species. To construct genomic libraries, large segments of genomic DNA are generated by random fragmentation, *e.g.*, using restriction endonucleases, and are ligated with vector DNA to form concatemers that can be

packaged into the appropriate vector. To prepare a library of cDNA from a specific cell culture, *e.g., Aspergillus niger*, mRNA is isolated from the culture and a cDNA library containing the gene transcripts is prepared from the mRNA.

The cDNA or genomic library can then be screened using a probe based upon the sequence of a known polynucleotide such as the polynucleotides cited here. Probes may be used to hybridize with genomic DNA or cDNA sequences to isolate homologous genes in the same or different plant species. In addition to probes derived from known polynucleotides, degenerate probes may be used. Techniques for making and using degenerate probes are well known in the art and can be found in Sambrook and Ausubel.

Alternatively, the polynucleotides of interest can be amplified from polynucleotide samples using amplification techniques. For instance, polymerase chain reaction (PCR) technology can be used to amplify the sequences of the genes directly from mRNA, from cDNA, from genomic libraries or cDNA libraries. PCR and other *in vitro* amplification methods may also be useful, for example, to clone polynucleotides that code for proteins to be expressed, to make polynucleotides to use as probes for detecting the presence of the desired mRNA in samples, for polynucleotide sequencing, or for other purposes.

Appropriate primers and probes for identifying ferulic acid esterase-specific genes, as well as xylanase sequences, from fungi and plant tissues are generated from comparisons of the sequences provided herein. For a general overview of PCR see PCR PROTOCOLS: A GUIDE TO METHODS AND APPLICATIONS, (Innis, M, Gelfand, D., Sninsky, J. and White, T., eds.), Academic Press, San Diego (1990). Reaction components are typically: 10 mM Tris-HCl, pH 8.3, 50 mM potassium chloride, 1.5 mM magnesium chloride, 0.001% gelatin, 200 µM dATP, 200 µM dCTP, 200 µM dGTP, 200 µM dTTP, 0.4 µM primers, and 100 units per mL Taq polymerase. Program: 96°C for 3 min., 30 cycles of 96°C for 45 sec., 50°C for 60 sec., 72°C for 60 sec, followed by 72°C for 5 min.

Polynucleotides may also be synthesized by well-known techniques as described in the technical literature. See, e.g., Carruthers, *et al., Cold Spring Harbor Symp. Quant. Biol.* 47:411-418 (1982), and Adams, *et al., J. Am. Chem.*

Soc. **105**:661 (1983). Double stranded DNA fragments may then be obtained either by synthesizing the complementary strand and annealing the strands together under appropriate conditions, or by adding the complementary strand using DNA polymerase with an appropriate primer sequence.

Suitable sources for the ferulic acid esterase used in this invention include but are not limited to, *Neurospora crassa, Aspergillus spp.* and specifically, *Aspergillus niger.* The xylanase used in this invention can be derived from any suitable source including, but not limited to, *Trichoderma reesei* and *Aspergillus spp.*

B. Preparation of Recombinant Vectors

To use isolated sequences in the above techniques, recombinant DNA vectors suitable for transformation of plant cells are prepared. Techniques for transforming a wide variety of plant species are well known and described in the technical and scientific literature. See, for example, Weising, *et al., Ann. Rev. Genet.* **22**:421-477 (1988). A DNA sequence coding for the desired polypeptide, for example a cDNA sequence encoding the full length FAE1 protein, will preferably be combined with transcriptional and translational initiation and targeting regulatory sequences which will direct the transcription of the sequence from the gene in the intended tissues of the transformed plant under the desired conditions.

Promoters can be identified by analyzing the 5' sequences of a desired gene. Sequences characteristic of promoter sequences can be used to identify the promoter. Sequences controlling eukaryotic gene expression have been extensively studied. For instance, promoter sequence elements include the TATA box consensus sequence (TATAAT), which is usually 20 to 30 base pairs upstream of the transcription start site. In most instances the TATA box is required for accurate transcription initiation. In plants, further upstream from the TATA box, at positions -80 to -100, there is typically a promoter element with a series of adenines surrounding the trinucleotide G (or T) N G. Messing, *et al.,* in GENETIC ENGINEERING IN PLANTS, pp. 221-227 (Kosage, Meredith and Hollaender, eds. (1983)).

A number of methods are known to those of skill in the art for identifying and characterizing promoter regions in plant genomic DNA (*see, e.g.,* Jordano, *et al., Plant Cell* **1**:855-866 (1989); Bustos, *et al., Plant Cell* **1**:839-854 (1989); Green, *et al., EMBO J.* **7**:4035-4044 (1988); Meier, *et al., Plant Cell* **3**:309-316 (1991); and Zhang, *et al., Plant Physiology* **110**:1069-1079 (1996)).

In construction of recombinant expression cassettes of the invention, a plant promoter fragment may be employed which will direct expression of the gene in all tissues of a regenerated plant. Such promoters are referred to herein as "constitutive" promoters and are active under most environmental conditions and states of development or cell differentiation. Examples of constitutive promoters include the cauliflower mosaic virus (CaMV) 35S transcription initiation region, the 1'- or 2'- promoter derived from T-DNA of *Agrobacterium tumafaciens*, the actin and ubiquitin promoters and other transcription initiation regions from various plant genes known to those of skill. A particularly preferred constitutive promoter is the rice actin promoter (see, McElroy, *Plant Cell*, **2**:163 (1990)).

Alternatively, the plant promoter may direct expression of the polynucleotide of the invention in a specific tissue (tissue-specific promoters) or may be otherwise under more precise environmental control (inducible promoters). Examples of tissue-specific promoters under developmental control include promoters that initiate transcription only in certain tissues, such as leaves, roots or seeds.

In one aspect of the instant invention, expression of FAE occurs after the the plant has been cut, removed from the ground or ingested. Thus an appropriate promoter would be a senescence promoter. For example, *BFN1* has recently been shown to be a nuclease expressed in senescing leaves, Perez-Amador, *et al., Plant Physiol.* **122**:169 (2000). Similarly, SAG12, a cysteine protease is also found in senescing leaves (Noh & Amasino, *Plant Mol. Biol.* **41**:181 (1999). In a preferred embodiment, the promoter from the *gem* gene of *Festuca pratensis* is used to direct expression of FAE in senescing leaves.

In another aspect, the FAE would be expressed upon ingestion by a foraging animal. Exemplary promoters for this aspect would include Soybean Gmhsp 17.5 promoter and the leucine aminopeptidase (LAP) promoter. The

GMhsp promoter is from a heat shock protein gene and initiates expression if the temperature of the environment is increased. In the laboratory, an increase of 15°C for 2 hours is the preferred heat shock. However, in non-laboratory conditions suitable increases in temperature will occur in silos and in the rumen of animals that have ingested the plants of this invention. The LAP promoter initiates the expression of the FAE gene upon wounding of the plant. Such wounding would occur after cutting the plant or after mastication by a foraging animal. Tissue specific promoters that could be used in this invention include promoters of genes that are differentially expressed in the leaves of grasses. An example of a leaf specific promoter is the *rbcS* promoter of tomato (*Proc. Nat'l Acad. Sci. USA* **84**:7104 (1987)). This promoter normally regulates a gene determined to be important in photosynthesis.

For proper polypeptide expression, a polyadenylation region at the 3'-end of the coding region should be included. The polyadenylation region can be derived from the natural fungal gene, from a variety of other fungal or plant genes, or from T-DNA. These sequences are well known and readily available to those of skill in the art.

In addition to a promoter and poly A+ sequences, the preferred expression vectors of this invention also will contain signal sequences. These are polynucleotides found at the 5' and/or 3' ends of the coding region and serve to target expression of the gene to specific cellular organelles. These signal sequences can be both upstream or downstream of the coding region. Some preferred examples of upstream signal sequences include the barley aleurain sequence (Rogers, *Proc. Nat'l Acad. Sci. USA* **82**:6512 (1985) which targets vacuoles and the Aspergillus apoplast signal. This signal sequence targets expression to the apoplast.

In addition to targeting expression to specific organelles, it may be desireable to retain the expressed FAE in the Golgi or endoplasmic reticulum. The well known ER retention signal, KDEL, can be added to the 3' end of the coding polynucleotide.

The vector comprising the expression cassettes (*e.g.*, promoters and/or coding regions) of the invention will typically comprise a marker gene which

confers a selectable phenotype on plant cells. For example, the marker may encode biocide resistance, particularly antibiotic resistance, such as resistance to hygromycin, kanamycin, G418, bleomycin, or herbicide resistance, such as *resistance to chlorosluforon or Basta.*

C. Production of Transgenic Plants

DNA constructs of the invention may be introduced into the genome of the desired plant host by a variety of conventional techniques. For example, the DNA construct can be introduced directly to plant tissue using ballistic methods, such as DNA particle bombardment or the constructs may be introduced directly into the genomic DNA of the plant cell using techniques such as electroporation and microinjection of plant cell protoplasts. Alternatively, the DNA constructs may be combined with suitable T-DNA flanking regions and introduced into a conventional *Agrobacterium tumefaciens* host vector. The virulence functions of the *Agrobacterium tumefaciens* host will direct the insertion of the construct and adjacent marker into the plant cell DNA when the cell is infected by the bacteria.

See Dalton et al. (Co-transformed, diploid Lolium perenne (Perennial Ryegrass), Lolium multiflorum (Italian Ryegrass) and Lolium temulentum (Darnel) plants produced by microprojectile bombardment. Plant Cell Reports (1999) 18(9), 721-726) for exemplary methods for culturing and transformation of grasses.

Microinjection techniques are known in the art and well described in the scientific and patent literature. The introduction of DNA constructs using polyethylene glycol precipitation is described in Paszkowski, *et al., Embo J.* 3:2717-2722 (1984). Electroporation techniques are described in Fromm, *et al., Proc. Natl. Acad. Sci. USA* **82**:5824 (1985).

Agrobacterium tumefaciens-mediated transformation techniques, including disarming and use of binary vectors, are well described in the scientific literature. See, for example Horsch, *et al., Science* **233**:496-498 (1984), and Fraley, *et al., Proc. Nat'l. Acad. Sci. USA* **80**:4803 (1983). US Patent 5,591,616 discloses *Agrobacterium* mediated transformation techniques in monocotyledons.

Ballistic transformation techniques are described in Klein, *et al., Nature*

327:70-73 (1987). In a preferred embodiment, a particle in-flow gun (PIG) is used to transform the plant cells of this invention.

Transformed plant cells which are derived by any of the above transformation techniques can be cultured to regenerate a whole plant that possesses the transformed genotype and thus the desired phenotype such as improved digestibility. Such regeneration techniques rely on manipulation of certain phytohormones in a tissue culture growth medium, typically relying on a biocide and/or herbicide marker which has been introduced together with the desired nucleotide sequences. Plant regeneration from cultured protoplasts is described in Evans, *et al.*, PROTOPLASTS ISOLATION AND CULTURE, HANDBOOK OF PLANT CELL CULTURE, pp. 124-176, Macmillian Publishing Company, New York, 1983; and Binding, *REGENERATION OF PLANTS, PLANT PROTOPLASTS*, pp. 21-73, CRC Press, Boca Raton, 1985. Regeneration can also be obtained from plant callus, explants, organs, or parts thereof. Such regeneration techniques are described generally in Klee, *et al., Ann. Rev. of Plant Phys.* **38**:467-486 (1987).

To determine the presence of or increase of FAE1 activity, an enzymatic assay can be used or an assay to measure increases and decreases in rates of fermentation. These assays are readily available in the literature and those of skill in the art can readily find them.

One of skill will recognize that other assays can be used to detect the presence or absence of FAE1. These assays include but are not limited to; immunoassays and electrophoretic detection assays (either with staining or western blotting).

The polynucleotides of the invention can be used to confer desired traits on essentially any plant. However, the main utility of this invention is in the improved digestibility of forage plants. Thus, it is envisioned the transgenic plants of this invention will include but not be limited to the following genera *Lolium, Festuca, Triticum, Avena,* and *Medicago*. The *FAE1* genes of the invention are particularly useful in the production of transgenic plants in the genus *Lolium.*

One of skill will recognize that after the expression cassette is stably incorporated in transgenic plants and confirmed to be operable, it can be

introduced into other plants by sexual crossing. Any of a number of standard breeding techniques can be used, depending upon the species to be crossed.

As mentioned above, the transgenic plants of this invention can be used as a foraging crop for animals, such as cattle, sheep, goats and horses. In addition, the methods of this invention can be used to transform any plant into which FAE expression is desired. For example, it is advantageous to break down cell walls during biomass conversion or during processing of plants for foodstuffs. This invention would help to achieve this goal more effectively and inexpensively.

The inventive methods herein may also be used to provide additional enzymes to enhance the availability of fermentable sugars in plants. Plant carbohydrates may be subject to further modification, either exogenously or endogenously, by the action of other enzymes. Such enzymes include, but are not limited to, endoglucanases, xylosidases and/or cellbiohydrolases. These enzymes may be provided either in an expression cassette provided for herein (i.e., endogenous) or applied to the plant cell walls (i.e., exogenous) to enhance the availability of mono- and/or di-saccharides.

Plants other than grasses may find a use in the present invention. For example, corn (or maize) is specifically contemplated to be useful. The grass Festuca is similar to maize in cell wall structure and therefore provides a good model of the ability to enhance fermentable carbohydrates in corn. Other useful plants contemplated for use in the present invention are Festuca, Lolium, Zea, Avena, Sorghum, Millet (tropical cereals), Miscanthus (a grass with potential for use as a biomass energy crop), Cenchrus, Dichanthium, Brachiaria and Paspalum (apomictic tropical range grasses) and Poa (Kentucky bluegrass).

Cell walls of forage grasses makes up 30-80% of forage dray matter representing a major source of energy for ruminants, but less than 50% of this fraction is digested by the animal. Conversion of low-value biomass to sugars and ethanol is also less than optimal due to the carbohydrate unavailability of the

feedstocks, including but not limited to bagasse, race straw, corn stover and corn fiber.

Ferulic and other hydroxycinnamic acids are ester linked to arabinosyl residues in arabinoxylans, and play a key role in crosslinking xylans to liginin, resulting in less degradable cell walls. Ferulic acid esterase (FAE) can release both monomeric and dimeric ferulic acid (FA) from arabinoxylans making the cell wall more susceptible to further enzymatic attack. Transgenic plants have been produced expressing an FAE gene following microprojectile bombardment of cell cultures. Measurements of the level of FAE activity from different vectors targeting FAE to the vacuole, ER and apoplast under constitutive or inducible (heat shock) promoters shows that at least for constitutive expression of vacuolar targeted FAE, the activity was highest in young leaves and increased along the leaf lamina. We also show that FAE expression results in release of monomeric and dimeric FA from cell walls on cell death and this was enhanced several fold by the addition of xylanase. An effect of FAE expression on the monomeric and dimeric cell wall ester linked ferulate content in comparison to control (non-transformed) plants is seen. Generally, the lower the levels of monomers and, in particular, dimers of hydroxycinnamic acids in leaves, the higher the digestibility and/or availability of complex carbohydrates for conversion.

Senescence is the terminal phase in leaf development and occurs without grouth or morphogenesis Therefore the metabolism/physiology of this stage of the leaf's lifespan can be targeted directly for alteration with minimal detrimental impact on early development. Senescence follows leaf maturity and is associated with the expression of specific genes. These genes and their controlling elements can be exploited to manipulate development, adaptation, productivity and quality traits in crop plants. There seems to be good conservation of senescence physiology across the range of higher plant species and thus these promoters are useful in the present invention.

The following preparations and examples are given to enable those skilled in the art to more clearly understand and practice the present invention. They should not be considered as limiting the scope and/or spirit of the invention, but

merely as being illustrative and representative thereof.

In the experimental disclosure which follows, the following abbreviations apply: eq (equivalents); M (Molar); μM (micromolar); N (Normal); mol (moles); mmol (millimoles); μmol (micromoles); nmol (nanomoles); g (grams); mg (milligrams); kg (kilograms); μg (micrograms); L (liters); ml (milliliters); μl (microliters); cm (centimeters); mm (millimeters); μm (micrometers); nm (nanometers); ° C. (degrees Centigrade); h (hours); min (minutes); sec (seconds); msec (milliseconds); Ci (Curies) mCi (milliCuries); μCi (microCuries); TLC (thin layer achromatography); Et (ethyl), Me (methyl).

Example 1
Preparation of Enzyme Encoding DNA Sequences

A genomic clone for FAE1 (see Figures 1-3) was used as the starting point for the preparation of an intronless FAE1 encoding DNA sequence. The sequence for the genomic clone is given in Figures 2 and 3. Separate fragments for both FAE exons were recovered by PCR from a 5.5kb EcoRI fragment of the genomic clone in pLITMUS28, and 'cDNA' created by overlapping PCR. See Figure 4.

Two 5' primers were used. FAE-S5 which amplifies the entire reading frame (including the Aspergillus signal), and FAE-N5 which amplifies only the mature protein (i.e. has *no* signal). A number of codons are optimised (underlined in primer sequences below). The overlap product may be derived from either FAE-I5 (wild type) or FAE-I3 (conserved Ser changed to Ala) primers, allowing production of enzymatically inactive protein to check toxicity. As shown in Figure 5, overlapping of PCR products made with FAE-I5 and FAE-I3 creates two possible uninterrupted reading frames. If the complement to FAE-I5 serves as the template when recombined then the encoded protein retains the serine moiety and the esterase is functional (highlighted serine is at active site). If the FAE-I3 primer serves as the template the serine is replaced with an alanine and the esterase is inactivated (highlighted alanine in bottom amino acid sequence given in Figure 5).

Where possible, codon usage has been optimised in constructed reading frames (codon choice based on published barley preferences).

FAE-I5 (SEQ ID NO: ___)
GGCGCCGAGGGAGTGGCCGGTCACGGTCAGCGCGTAGTCC 40-mer
FAE-I3 (SEQ ID NO: ___)
CCGGCCACGCCCTCGGCGCCTCCCTGGCGGCACTC 35-mer
FAE-N5 (SEQ ID NO: ___)
CTAAAGCTTACCATGGCGGCCGCCTCCACGCAGGGCATCTCCGA 44-mer
FAE-S5 (SEQ ID NO: ___)
CTAAAGCTTAACATGAAGCAGTTCTCCGCCAA 32-mer
FAE-3 (SEQ ID NO: ___)
TCTAAGCTTGCGGCCGCGACCGGCCAGGTGCATGCGCCGCTCGTCATCCC
50-MER

Example 2
Preparation of Vectors

Vectors had the general structure shown in Figure 6.

A. Plant transformation vector series

Initial expression vectors were based on **pCOR105** [rice actin promoter - McElroy et al. MGG 231:150-160 (1991)] (Figure 7). pCOR105 Not and SstII sites were first destroyed [cut with NotI and SstI, followed by heat inactivation and T4 DNA polymerase treatment in the presence of dNTPs] using standard methods as described in Maniatis et al. or following the manufacturer's instructions for enzymes to simplify subsequent Not cassette manipulation and allow use of unique Sst site (see below).

The *nos* terminator from **pMA406** (Ainley & Key (1990) PMB 14:949-60) was amplified by PCR using primers TER5 and TER3 to generate a fragment with the following sequence (SEQ ID NO:___):

(Pst1) (Not 1)
(AGACTGCAGACCATGGCGGCCGCGKAACCACTGAAGGATGAGCTGTAAAG
AAGCAGATCGTTCAAACATTTGGCAATAAAGTTTCTTAAGATTGAATCCTGTT
GCCGGTCTTGCGATGATTATCATATAATTTCTGTTGAATTACGTTAAGCATGT
AATAATTAACATGTAATGCATGACGTTATTTATGAGATGGGTTTTTATGATTA
GAGTCCCGCAATTATACATTTAATACGCGATAGAAAACAAAATATAGCGCGC
AAACTAGGATAAATTATCGCGCGCGGTGTCATCTATGTTACTAGATCGATA
AGCTT CTA GATCT (where **K**=G or T)
(HindIII) (XbaI)

A redundancy in the TER5 primer (GCGKAA) creates fragments having either a stop codon (TAA) or glutamate codon (GAA) in one reading frame. The glutamate codon is in frame with a downstream KDEL motif.

The fragment and modified pCOR105 vector were cut with PstI and XbaI, according to manufacturers instructions, relevant fragments gel-purified, ligated with T4 DNA ligase and transformed into *E. coli*. Resulting clones were then sequenced to establish which TER5 alternatives were present.

Initial FAE expression vectors were then constructed from these vectors by inserting FAE-S5/FAE-3 PCR products (T4 DNA polymerase 'polished' in the presence of dNTPs, purified and digested with NotI, cloned into EcoRV and NotI digested vector) or FAE-N5/FAE-3 PCR products (purified and NotI digested, cloned into NotI digested and calf intestinal alkaline phosphatase treated vector).

The initial pCOR105-*nos* terminator clones were also modified by the addition of ALE-5/ALE-3 PCR products (encoding wild-type and modified barley aleurain signal peptides, see below for details). The products were 'polished' with T4 DNA polymerase in the presence of dNTPs, purified and cut with NotI, then cloned into EcoRV and NotI digested vectors. Addition of the ALE sequences creates a series of vectors which can express a reading frame inserted at the NotI or NcoI sites as a fusion to the barley aleurain signal, with or without vacuolar targetting motif, and with or without an ER retention motif. HindIII sites flanking the translation initiation codon and transcriptional terminator allow easy

movement of transcription units between expression vectors providing different promoter sequences. (See Figure 8 depicting the generic ALE-TER vector.)

Vector sequences were confirmed by sequencing. Two artifacts were found. Firstly, the redundant codon in TER5 was found to be AAA in one clone, which was subsequently used as the source of all KDEL fusions (ie peptide sequence is KPLKDEL, rather than EPLKDEL as designed). See Figure 9. Secondly, an additional base is found at the site of the redundant codon in one clone, creating a frameshifted terminal peptide (ETTEG, Figure 10) which was used as a control in some constructs.

Exploitation of the modular arrangement of signal peptides in the above vector series allowed various combinations of FAE and targeting motifs to be created using standard molecular biology procedures (i.e., restriction digest, purification of relevant fragments and ligation as appropriate). For example, the NotI fragment containing the FAE reading frame was inserted into the NotI site of the frameshifted clone described above to create vector **pTP3.1**. The native Aspergillus COOH-terminus was inserted into a FAE-S5/FAE-3 clone as a SphI (T4 DNA polymerase polished) – NcoI fragment from the FAE genomic clone (replacing the NotI (T4 DNA polymerase polished) – NcoI fragment), creating vector **pTP4a2**, which then encodes the entire, unmodified, Aspergillus FAE. Replacement of the SalI/XbaI fragment of pTP3.1 with that of pTP4a2 then created **pTP11.1**, which encodes FAE with a native Aspergillus COOH-terminus but a barley aleurain N-terminal signal.

Briefly, other vectors made in this series were; **pTP8.5**, the FAE NotI fragment inserted into the NotI site of an ALE-frameshifted COOH-terminus construct, aleurain N-terminus; **pTP5.1**, replacement of the native Aspergillus COOH terminus with a KDEL peptide (NotI/XbaI fragment exchange), Aspergillus N-terminal signal retained; **pTU4.4**, BamHI fragment of pTP11.1 replaces BamHI fragment of pTP5.1, creates FAE reading frame fused to heterologous N- and C-termini (aleurain signal and KDEL).

Vectors in which the aleurain vacuolar targeting motif NPIR was replaced by NPGR (found to be inactive in some plant assays) were created by replacing an EcoRV/NotI fragment with ALE PCR product which had been cut with AccI

(T4 DNA polymerase polished) and NotI (vectors **pTT5.5** and **pTT5.14**, Aspergillus COOH-terminus). The BamHI fragment of pTT5.5 was used to replace that of pTP5.1 to produce **pTU5**, creating an FAE reading frame fused to heterologous N- and C-termini (NPGR modification of aleurain signal and KDEL). The aleurain signal was also modified by PCR mutagenesis to remove the vacuolar targeting NPIR motif in its entirety (directed by primer ALECUT, which contains a NotI site to allow exchange of BglII/NotI fragments). NPIR deletion was created in this way in pTP11.1 (creating **pUA4.4**), and in pTP5.1 by exchange of BamHI fragments with pUA4.4 (creating **pUG4**).

Finally, PCR mutagenesis, using overlap of fragments generated by primers GLY3 and GLYB, was also used to alter a potential glycosylation site (asparagine codon changed to aspartate, as carried out for example in Chen, H.M., C. Ford & P. J. Reilly (1994) Biochem J 301 275-281 Substitution of asparagine residues in Aspergillus awamori glucoamylase by site-directed mutagenesis to eliminate N-glycosylation and inactivation by deamidation; see sequence data for exact change, vector **pTP10.1**).

PCR primers
TER-5 (SEQ ID NO:___)
AGACTGCAGACCATGGCGGCCGCGKAACCACTGAAGGATGAGCTGTAAAG AAGCAGATCGTTCAAACATTTG 72-MER (The KDEL stop codon is underlined.)
TER-NOT (SEQ ID NO:___)
AAGACTGCAGACCATGGCGG 20-MER
TER-3 (SEQ ID NO:___)
AGATCTAGAAGCTTATCGATCTAGTAACATAGATGACACC
ALECUT (SEQ ID NO:___)
CTAGGCGGCCGCGCGGGAGGAGGCGACGGCGAC
GLYB (SEQ ID NO:___)
GAGGGTGTATTCGGTATCGAGTTGCAGGTTCGTATC
GLY3 (SEQ ID NO:___)
CTCGATACCCATTACACCCTCACGCCTTTCGA

B. Construction of different promoter vectors

Various promoters were used to optimize expression and to establish constitutive, heat-shock inducibility and senescence enhancement.

i. Rice actin promoter and 1^{st} intron

Initial vectors (Figures 11 and 12) were constructed from pCOR105 which was subsequently found to contain a 5bp deletion relative to the published sequence which destroys the AccI site (GTAGGTAGAC, deleted bases underlined) and may affect splicing at the adjacent 3' site. The original rice actin sequence in this region (GTAGGTAG) was therefore restored using oligonucleotide **NCO**-ACT (CTCACCATGGTAAGCTTCTACC TACAAAAAAGCTCCGCA) by replacing the BglII/HindIII fragment with a PCR product, to produce vector **pPQ10.1**.

A rice repetitive element is present in the upstream region of the actin promoter used in **pCOR105**; as this may have unpredictable effects on vector expression it was removed from **pPQ10.1** by deletion of the KpnI/EcoRI fragment (end-filled with T4 polymerase and ligated following digest, restoring EcoRI but not KpnI), to produce vector **pGT6.** The HindIII fragment containing the FAE reading frame and nos terminator of **pTP3.1** (see Example 2A) was then inserted into pGT6 to produce construct **pJO6.3.**

ii. Soybean heat-shock promoter

A soybean heat shock promoter from a 23kD HSP was obtained from pMA406 (Ainley & Key (1990) PMB 14:949-60). This promoter when fused to β-glucuronidase (Jefferson et al 1987 EMBO J 6:3901-3907) had previously been shown to be inducible by a 10°C heat-shock and show stable expression for 24-48 hours (data not shown). β-glucuronidase fusions are a sensitive and versatile fusion marker in higher plants. The construction of the co-integration HS vectors is given below.

iii. Senescence enhanced expression (See1) promoter from Lolium multiflorum

The promoter and signal sequence (including NPIR motif) of the LSee1 gene was amplified from *Lolium multiflorum* cv Tribune with oligonucleotides SEE-NCO and SEE-VAC, and cloned as an Asp718/NotI replacement of the promoter region of vector **pTP11.1**. Following sequencing to screen for PCR artifacts, one of three identical clones was chosen **(pUB8.11)**.

The See1 promoter from maize has been cloned previously and has

EMBL accession number is AX050343. See WO0070061.

The Lolium version of See1 was also cloned previously (Qiang Li (2000) Studies on leaf senescence and its genetic manipulation in *Lolium mutiflorum* PhD Thesis University of Wales, Aberystwyth) and has been shown to be senescence inducible when used to drive both β-glucuronidase and the Agrobacterium ipt gene.

An apoplast-targeted derivative was constructed by amplifying the Potato Protease Inhibitor (PPI) motif with primers PPI-AP6 and SEE-ATG, and cloning the product as an NgoMIV/NotI fragment into pUB8.11 (NgoMIV partial digest), to produce vector **pJQ5.2.** This vector has both the senescence induced promoter and the apoplast target sequence with the gene to be expressed inserted downstream of the apoplast sequence.

PCR Primers

SEE-VAC (SEQ ID NO: ___)

AACCATGGCGGCCGCGCGCTCGGTGACGGGCCGGAT

SEE-NCO (SEQ ID NO: ___)

TTCGGTACCATGGCCAGGTATAATTATGG

SEE-ATG (SEQ ID NO: ___)

CTGCGCCGGCGAGATGGMCGTGCACAAGGAG

C. Construction of targeting sequences

In order to examine whether or not the localization of the enzyme would have an effect on the phenolic acid content of the cell wall various signal sequences were utilized. The targeting sequences were added either to the N-terminus or to the C-terminus of the gene of interest.

i. N-terminal signal sequences

Six N-terminal signal sequences were utilized:

(a) The native Aspergillus end of FAE, plus excretion signal [apoplast localisation]

This is from the original clone and has the peptide sequence: MKQFSAKHVLAVVVTAGHALAASTQGI.

(b) The mature Aspergillus end, with no excretion signal [cytoplasmic localisation]

Peptide sequence is MAAASTQGI (underlined motif is common to all constructs). Truncation of the signal sequence in (a) above was carried out by PCR with mutagenic primer FAE-N5.

(c) The barley aleurain signal, including intact NPIR motif [vacuole localisation]

The barley aleurain vacuolar signal sequence (See Figure 13; Swissprot database accession number P05167) was derived entirely from overlapping primers (ALE-5, ALE-3, ALE-CUT ALE-CAP-5 and ALE CAP-3). Following primer annealing at 37°C and extension with T4 DNA polymerase in the presence of dNTPs according to manufacturers instructions, PCR with flanking primers ALE-5 and ALE-3 was carried out. The product was 'polished' with T4 DNA polymerase, purified, digested with NotI and cloned into EcoRV/NotI digested pCOR105-nos terminator vector (see above). ALE-3 contains redundancies so that clones encoding NPIR or NPGR motifs may be recovered. Two versions of the signal, with and without the vacuole targeting motif, were produced, to give putative vacuolar NPIR and apoplast (NPGR) signal sequences.

PCR Primers

ALE-5 (SEQ ID NO: ___)

GGAATTCGTAGACAAGCTTACMATGGCCCACGCCCGCGTCCT 41-MER

ALE-3 (SEQ ID NO: ___)

TATCCATGGCGGCCGCGCGGTCGGTGACGGGCCGGMYCGGGTTGGAGTCGGCGAA 55-MER

ALE-CUT (SEQ ID NO: ___)

CTAGGCGGCCGCGCGGGAGGAGGCGACGGCGAC 33-mer

ALECAP-5 (SEQ ID NO: ___)

GCGACGGCGACGGCGGCCGTGGCCAGCACGGCGAGCGCCAGGAGGAGGACGCGG 54-MER

ALECAP-3 (SEQ ID NO: ___)

TCGCCGTCGCCTCCTCCTCCTCCTTCGCCGACT 33-MER

(d) The barley aleurain signal, mutated to a NPGR motif [cytoplasmic localisation]

(e) The rat sialyl transferase golgi targeting motif [golgi localisation]

A Golgi targeting vector, **pJQ3.2**, was made by inserting a reading frame encoding the relevant rat sialyl transferase (RST) motif (See Figure 14. RST motif shown to function in plants by Boevink P, Oparka K, Cruz SS, Martin B, Betteridge A, Hawes C, (1998) PLANT JOURNAL 15 441-447 Stacks on tracks: the plant Golgi apparatus traffics on an actin/ER network) into vector pPQ10.1, and replacing the EcoRI/NotI promoter/signal fragment of **pJO6.3** with the fragment from this vector. Briefly, the RST motif was constructed by annealing oligonucleotides RST-F1A, RST-F1B, RST-F2A and RST-F2B, and amplifying the product with RST-5AD and RST-3A. This product was cloned and sequenced. Clones were found to have a deletion which was corrected by PCR with RST-RPT, followed by overlap-PCR and cloning of products.

PCR primers

RST-5AD (SEQ ID NO: ___)

ACTAAGCTTAAGGAGATATAACAATGATCCACACCAACCTCAA

RST-F1A **(SEQ ID NO: ___)**

TTCCATGATCCACACCAACCTCAAAAAGAAGTTCTCCCTCTTCAT

RST-F1B **(SEQ ID NO: ___)**

AGAGTGATCACGGCGAAGAGGAGGAAGACGAGGATGAAGAGGGAGAACTTCTTTT

RST-F2A **(SEQ ID NO: ___)**

TATAGATCTGCGTGTGGAAGAAGGGCTCCGACTACGAGGCCCTCACCCTCCAAGCCAAGGA

RST-F2B **(SEQ ID NO: ___)**

CATTTGGAACTCCTTGGCTTGGAGGGTG

RST-3A **(SEQ ID NO: ___)**

AACCATGGCGGCCGCCATTTGGAACTCCTTGGCT

RST-RPT **(SEQ ID NO: ___)**

TATAGATCTGCGTGTGGAAGAAGGGCTCCGACTACGAGGCCCTCACCCTCC AAGCCAAGGA

(f) otif [cytoplasmic localisation]

(g) The potato protease inhibitor II (PPI) apoplast motif [apoplast localisation]

An apoplast targeting reading frame was designed to encode the relevant potato protease inhibitor II (PPI) motif (See Figure 15) and cloned into **pJO6.3**, to produce vector **pJQ4.9.** Briefly, the PPI motif was constructed by annealing oligonucleotides PPI-AP1, PPI-AP2, PPI-AP3, PPI-AP4, PPI-AP5 and PPI-AP6, and cloning this product as a HindIII/NotI fragment into vector pPQ10.1; the EcoRI/NotI promoter/signal fragment of pJO6.3 was then replaced with the equivalent fragment from the modified pPQ10.1 vector.

PCR primers

PPI-AP1 **(SEQ ID NO: ___)**

GGAATTCGTAGACAAGCTTACMATGGMCGTGCACAAGGAGGT

PPI-AP2 **(SEQ ID NO: ___)**

GATCAGGAGGTAGGCWACGAAGTTWACCTCCTTGTGC

PPI-AP3 **(SEQ ID NO: ___)**

CCTACCTCCTGATCGTSCTCGGCCTCCTCTTGCTCGT

PPI-AP4 **(SEQ ID NO: ___)**

CCTTGGCGTCCACGTGCTCCATGGCGGAWACGAGCAAGAGGAG

PPI-AP5 **(SEQ ID NO: ___)**

GTGGACGCCAAGGCCTGCACCCKCGAGTGCGGCAACCTC

PPI-AP6 **(SEQ ID NO: ___)**

GGAATTCGCGGCCGCCGGGCAGATGCCGAAGCCGAGGTTGCCGCACT

ii. C-terminal end signal sequences

Four C-terminal signal sequences were utilized:

(a) Native Aspergillus end, [CTW] (vacuole and apoplast vectors)

This was derived directly from the genomic clone (see Example 1) as a Nco1-Sph1 fragment (Sph end filled with T4 polymerase) which replaces the

Nco1-Not1 region of a standard actin -FAE vector (Not1 end filled with T4 DNA polymerase).

(b) Expression vector linker alone [CTW-PVAAA] (plant optimised C-terminus for vacuole, golgi and apoplast vectors)

. CTW is the peptide sequence of the Aspergillus FAE COOH end and is here provided by oligo FAE3. In this primer the reading frame is extended to provide the additional amino acids PVAAA which are partially encoded by the Not1 site used for cloning downstream signals see c) and d) below. Some COOH amino acids /motifs may affect compartment targeting, the PVAAA sequences are expected to be neutral in this respect while the native Aspergillus end may not be.

(c) Linker plus KPLKDEL [first K is primer artifact, intended to be E] (ER retention vectors)

These sequences are provided by primer TER5 introduced during PCR to generate the nos terminator fragment, and identified by sequencing within a specific clone . KDEL targeting has been demonstrated in plants by Denecke et al. ((1992) EMBO J 11: 2345-2355 Plant and mammalian sorting signals for protein retention in the endoplasmic reticulum contain a conserved epitope).

(d) Linker plus ETTEG [frameshift of (c)] (loss of ER retention - vacuole vectors)

These sequences are provided by primer TER5 introduced during PCR to generate the nos terminator fragment, and identified by sequencing within a specific clone (see Example 2A).

The KDEL signal is for ER retention, while others provide controls. A frameshift in the TER5 region [additional A] was used in subsequent constructs to destroy the ER KDEL retention signal.

The linker used in the above C-terminal targeting sequences was PVAAA.

D. Co-integration and co-transformation vectors.

Co-transformation vectors

A Hygromycin resistance gene driven by a CaMV345S promoter **(pRob5)** (35S-HYG-CMV in pUC18 (modified HYG, derived from pGL2) Bilang et al (1991)

Gene 100:247-50) was used for co-transformation experiments with **pTT3** and **pTP3.1, pJQ4.9, pJQ3.2, pJO6.3, pJQ5.2, pUB8.1 1** vectors.

Co-integration vectors

1. **Actin promoter constructs - pTR2.22, pTR6.1 , pTR8.1, pTR9.4, pTR7.1, pTT5.5 and 5.1.**

The CAMV35S-hyg region from pAJEB64TCA [a plant expression vector constructed by Andy Bettany at IGER containing CaMV-HYG from pTRA151 (Zheng et al 1991 Plant Physiol 97:832-835) (CaMV35S-HYG-tml terminator as clonable cassette in pUC4) cloned into KpnI site of pCOR105] was added as a HindIII fragment at the KpnI site (T4 polymerase blunt) of pTP4a2, in divergent orientation to FAE to create **pTR2.22.** The FAE/Nos HindIII fragment of this vector was replaced as follows in co-expression vectors . From pTP5.1 for **pTR6.1** , from pTP10.1 to **pTR8.1**, from pTP11.1 to **pTR9.4.** Signal sequences of FAE in pTR2.22 were replaced as HindIII/BglII fragments in **pTR7.1** (fragment from pT09.1). PCR products (ALE5/ALE-G) was digested with Acc1 and T4 polymerase, polished, followed by Not1 digest and cloning into EcoRV/ Not1 digested pTR2.22 to give clones **pTT5.5 and 5.1.**

PCR primer
ALE-G
TATCCATGGCGGCCGCGCGGTCGGTGACGGGCCGGCCCGGGTTGGAGTC
GGCGAA

2. **Actin promoter constructs -pUF1, pUA1K3, pUH4, pUH5, pUH6, pUH7, pUH8, pUH9.**

The HygR gene from pAJEB64TCA, driven by the CaMV promoter, was first cloned as an end-filled HindIII fragment at the end-filled XbaI site of pTP3.1, to give **pHOX3.** For ease of cloning the downstream HindIII site was destroyed to create **pUA1K3** and replacement of the FAE/Nos terminator HindIII fragment in this vector was carried out as follows. From pTP5.1 for **pUF1**, from **pTP11.1** for pUH4, from pTP8.5 for **UH5,** from pTT5 for **pUH6**, from pUA4.4 for **pUH7**, from pTU5 for **pUH8** and from pUG4 for **pUH9.**

3. **Heat-shock promoter constructs - pUH10, pUH12, pUC5.11.**

A co-transformation vector in which FAE is expressed from the soybean heat shock promoter was made by first modifying pMA406 to remove the nos terminator (BglII linearised and gel purified, KpnI digested, T4 DNA polymerase polished in the presence of dNTPs and recircularised), and then inserting the FAE HindIII fragment from pTP11.1, creating pTT3.1, which encodes the full aleurain signal and the native Aspergillus COOH-terminus.

Following assays of various constructs, co-integration vectors were constructed with FAE and HygR genes arranged in tandem.

The HygR gene from pAJEB-64-TCA, driven by the CaMV promoter, was first cloned as an end-filled HindIII fragment at the end-filled XbaI site of pTP3.1, to give pHOX3 and subsequently excised as a HindIII/SacI fragment (partial SacI digest, relevant sites found in flanking pTP3.1 sequences) which was cloned into the HindIII/SacI sites of pMA406, in tandem orientation (vector **pUH1a20**). FAE sequences were then cloned into the HindIII site of **pUH1a20** downstream of the heat -shock promoter (HindIII fragment from pTU5 for **pUH10**, HindIII fragment from pTT5 for **pUH12**). A pTP3.1 derivative was made by cloning the CaMV/HygR HindIII cassette from pAJEB-64-TCA in tandem orientation downstream of the FAE gene in pTP3.1, inactivating the middle HindIII site by partial digestion and end-filling, and excising the combined FAE/HygR cassette as a single HindIII fragment, which was inserted at the HindIII site in pMA406 to produce **pUC5.11**.

Example 3

Transformation of Plant Cells

Eight to ten weeks old embryogenic *F. arundinacea* and *L. multiflorum* suspension cultures were bombarded either with a single co-integration plasmid DNA vector containing FAE and hyg resistance genes, or with a co-transformation vector containing FAE and with plasmid pROB5 conferring hygromycin resistance (CAMV35S-hpt- nos) using a Particle Inflow Gun (PIG) (Finer et al. (1992) Development of the particle inflow gun for DNA delivery to

plant cells Plant Cell Reports 11:323-328) and 1.5-3.0 µm gold particles as in Dalton *et al* (Dalton et al. (1999) Co-transformed diploid *Lolium perenne (Perennial ryegrass), Lolium multiflorum* (Italian ryegrass*) and Lolium temulentum* (Damel) plants produced by microprojectile bombardment. Plant Cell Reports. 18: 721-726) and Kuai et al (Regeneration of fertile transgenic tall fescue *(Festuca arundinacea)* plants with a stable highly expressed foreign gene. Plant Cell Tissue and Organ Culture (1999) 58:149-154). Transformants were selected with hygromycin (25 to 50mg /l) over a 10-12 week selection period at 25°C under continuous white fluorescent light (60 µE m^2 s^{-1}) and plants regenerated via somatic embryogenesis as in Dalton *et al* 1999, *supra*. Regenerated plants were screened for FAE activity on transfer to soil and expressing plants grown to maturity in a containment growth room at 18°C under 16h fluorescent lights (350 µE m^2 s^{-1}). Mature plants (6-8 weeks old) were re-assayed for FAE activity and fresh tissue harvested for Southern, Northern and Western analysis, and for self digestion analysis. The remaining tissue was freeze dried and powdered for cell wall structure analysis, in vitro-dry matter digestibility (IVDMD) determinations and for in-vitro gas production determinations of rates of tissue digestion.

Example 4

Targeting of Expression Product

To verify that the targeting sequences are effective in delivering the gene the targeting sequences were operably linked to a green fluorescent protein GFP. The vector constructs are shown in Figure 16. Cells were transformed by particle bombardment as in Example 3. Localization of the GFP could be visualized under a microscope 1 day after bombardment (i.e., shooting). See Figure 16.

Example 5

FAE1 activity

Plants regenerated from transformed cells showed FAE activity in all plant tissues tested. Cells were transformed as above under the direction of the ER and APO targeting sequences. FAE activity in transformed *Festuca arundinacea*

leaves of different ages was elevated compared to control (untransformed) plants. See Figures 17 and 18.

Similar results were seen with Lolium mutiflorum leaves at different ages transformed as above under the direction of vacuolar, ER and APO targeting sequence. See Figures 19 and 20.

FAE expression under a heat shock promoter can also be induced. (Data not shown.)

Thus, we have demonstrated FAE expression in Festuca and Lolium leaves under constitutive and HS promoters with effective FAE targeting to the vac, ER and apo.

FAE assay

FAE activity was determined in soluble extracts of fresh (or frozen at -70oC) leaves or cell cultures (0.5g) with 0.1M NaAc, pH 5.0 extraction buffer. Extracts were incubated with 24mM EF (ethyl 4-hydroxy-3-methoxycinnamate) or 1% FAXX as substrate, at 28°C for 24hrs and FAE activity calculated as the amount of ferulic acid released. FAE activity was also determined by measuring the release of monomeric and dimeric ferulic acid from self-digested leaf or cell culture samples. Fresh, or frozen, leaves or cell cultures (0.5g) were ground in 0.1M NaAc, pH5.0 extraction buffer in the presence and absence of xylanase (1000U **GC140**/sample) without added substrate and incubated at 28°C for 72hrs. Following incubation, and centrifugation, soluble extracts were loaded onto an activated reverse phase C18 µNova sep-pak column (Waters), eluted with 100% MeOH and the MeOH sample analysed by HPLC.

Example 6

Chemical Analysis of Cell Wall Extracts

Ester bound compounds were extracted from freeze dried powdered leaves or cell cultures (50 -100mg) with NaOH (5ml of 1M) followed by incubation at 25°C for 23hrs under N2. After centrifugation and acidification of the soluble extract with concentrated HCl, the extracted phenolics were loaded onto an activated reverse phase C18 µNova sep-pak column (Waters) and eluted with 100% MeOH. and the MeOH sample analysed by HPLC.

HPLC was carried out with methanol: 5% acetic acid either with a 35-65% MeOH gradient in 15min (FAE assay) or with a 30-70% MeOH gradient in 25 min (monomer and dimer cell wall components) at 2ml/min on a μNova Pak C18 8x10 RCM (Waters). Extracts were detected and quantified with a diode array detector (240-400nm Waters 996PDA) monitored at 280nm for aldehydes and 340nm for hydroxycinnamic acids. .

Levels of esterified monomeric and dimeric hydroxycinnamic acids in Festuca arundinacea plants expressing FAE under VAC, and ER and APO targeting sequences are reduced compared to control (untransformed) plants. The results can be seen in Figure 21 and 22, respectively. Thus, we show where this does not result in reduced cell wall phenolics in growing plants with vac targeting but does result in lower phenolics with ER and apo targeting. In addition,

Levels of esterified monomeric and dimeric hydroxycinnamic acids in Festuca arundinacea plants expressing FAE are not significantly reduced when FAE is VAC, targeting (Fig 21) which is as predicted for correct vacuolar targeting, but are significantly reduced, as predicted, in some plants when FAE was ER and APO targeted, compared to control (untransformed) plants. The results can be seen in Figure 22.

Example 7

In vitro dry matter digestibility. (IVDMD)

The *in vitro* dry matter digestibility (IVDMD) was estimated on 1.0 g dry weight of leaf or cell culture tissue using the pepsin/cellulase method of Jones and Hayward (The effect of pepsin treatment of herbage on the prediction of dry matter digestibility from solubility in fungal cellulase solutions. Journal of the Science of Food and Agriculture (1975) 26:711-718).

We show that the presence of FAE in the plants results in higher digestibility of the leaves. This may be due to internal FAE activity acting on normal cell walls with vacuole located FAE and to both FAE activity and the lower cell wall crosslinking with ER and apo targeted FAE (as also found with cell cultures).

End point digestibility as determined by IVDMD were higher in leaf tissue of some transformed plants of Festuca expressing FAE, compared to control (untransformed) plants. Examples are shown where vacuolar, ER or apoplast targeted FAE under a constitutive actin promoter have been effective at increasing IVDMD. Similar results were obtained with in leaves of Lolium, but were less pronounced.

The results can be seen in Figures 23 and 24.

Example 8

In vitro gas production measurements

In each experiment, 1.0-g samples of freeze dried powdered leaf tissue or cell culture were fermented in three 165-ml capacity serum bottles according to the pressure transducer technique of Theodorou et al. (Theodorou et al. (1994) A new gas production method using a pressure transducer to determine the fermentation kinetics of ruminant feeds. Animal Feed Science and Technology 48: 185-197). Grab samples of rumen-digesta were taken at 8.00 h before the morning feeding from fistulated wethers fed grass hay, and transported to the laboratory in a pre-warmed (39°C) vacuum flask. The microbial inoculum and culture media were prepared as described by Theodorou et al. (1994). Each serum bottle received 10 ml of microbial inoculum, 85 ml of buffer and 4 ml of reducing agent.

At the end of the incubation period, (144h) the contents of each serum bottle were filtered through pre-weighed sintered glass funnels and freeze dried to constant weight. Dry matter loss was calculated as the difference between the dry weight of the sample pre- and post-incubation. Additionally, the concentration of volatile fatty acids (VFA) in the liquid fraction of the culture media at the end of the 144-h incubation period was determined by gas chromatography. A Chrompack CP 9000 chromatograph fitted with an automatic sampler (Chrompack 911) and a flame-ionisation detector, linked to a Dell PC with A1-450 integration software, was used for VFA quantification.

Gas production data were fitted to the model of France et al. (France, J., Dhanoa, M.S., Theodorou, M.K, Lister, S.J., Davies. D.R. and Isac, D. 1993. A model to interpret gas accumulation profiles associated with *in vitro* degradation of ruminant feeds. *Journal of Theoretical Biology.* 163: 99-111.) using the MLP (Ross,

G.J.S. 1987. *MLP, Maximum Likelihood Program Version 3.08.* Oxford Numerical Algorithms Group) package. The equation is in the form, $Y = A\{1 - e^{[-b(t-T) - c(\sqrt{t}-\sqrt{T})]}\}$ where Y is the cumulative gas production (ml), A is the asymptote (i.e. gas pool), T is lag time, and b (h^{-1}) and c ($h^{-0.5}$) are decay rate constants. A combined fractional rate (h^{-1}) of gas production (μ) was calculated as, $\mu = b + c/2\sqrt{t}$, where t is the incubation time (h).

It can be seen for *Festuca arundiancea* (denoted as BN in Figure 25) that cell cultures have a higher rate of digestion and cumulative gas production in the presence of FAE and that the addition of an exogenous xylanase further enhance the availability of fermentable carbohydrates. Similar results are found in FAE expressing cultures without added FAE . Fermentation rates are further increased compared with controls by the addition of exogenous FAE or xylanaase as these cultures expressing FAE have a reduced cell wall phenolic composition to controls Figures 26-28.

Example 9
FAE & xylanase transformed plants

Addition of exogenous xylanase (GC140) greatly increased FAE mediated release of phenolics from *Festuca* and *Lolium* leaves expressing *A. niger* FAE. See Figures 29-31 which show that phenolic release from leaf cell walls is increased in all FAE expressing plants on cell death and this is stimulated by xylanase irrespective of the targeting. Therefore expression of a fungal xylanase in plant cells is tested.

The FAE expression cassette is modified to comprise a fungal xylanase gene (either *T. reesei* or *A. niger*) to yield a FAE-xylanase expression cassette. The FAE-xylanase expression cassette is used to transform plant cells in a manner similar to those described in Example 3. The transformed cells are allowed to grow and are selected on an appropriate medium. The enzymes so expressed increase the availability of fermentable carbohydrates to a greater extent than the FAE expression cassette.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity and understanding, it will

be obvious that certain changes and modifications may be practiced within the scope of the appended claims.

What is claimed:

1. A transgenic plant comprising an expression cassette comprising a promoter operably linked to a ferulic acid esterase encoding polynucleotide.

2. The plant of claim 1, wherein the polynucleotide is derived from *Aspergillus niger.*

3. The plant of claim 2, wherein the polynucleotide is FAE ! from *Aspergillus niger.*

4. The plant of claim 3, wherein the polynucleotide encodes a ferulic acid esterase with an altered glycosylation site.

5. The plant of claim 3, wherein the polynucleotide encodes a ferulic acid esterase with a substitution so that glycosylation is altered.

6. The plant of claim 3, wherein the polynucleotide further comprises a polynucleotide that encodes CTWPVAAA at the 3' end.

7. The plant of claim 3 wherein sub-optimal codons are modified to *Triticum spp.* preferred codons.

8. The plant of claim 1, wherein the introduction of the ferulic acid esterase polynucleotide into the plant is by sexual reproduction.

9. The plant of claim 1, wherein the promoter is an inducible promoter.

10. The plant of claim 9, wherein the promoter is a senescence promoter.

11. The plant of claim 9, wherein the promoter is a heat shock promoter.

12. The plant of claim 1, wherein the promoter is a constitutive promoter

13. The plant of claim 1, wherein the expression cassette further comprises a polynucleotide sequence that targets expression of the

polynucleotide.

14. The plant of claim 13, wherein the polynucleotide sequence is upstream of the N-terminus of the ferulic acid esterase polynucleotide.

15. The plant of claim 14, wherein the polynucleotide is derived from the signal sequence of a vacuolar targeted gene

16. The plant of claim 15, wherein the targeted gene is a barley aleurain gene.

17. The plant of claim 15, wherein the vacuolar signal sequence of the polynucleotide is modified to produce a endoplasmic reticulum or apoplast signal sequence.

18. The plant of claim 15, wherein the polynucleotide is derived from the signal sequence of a vacuolar targeted senescence gene.

19. The plant of claim 18, wherein the senescence gene is a Lolium *See1* signal sequence.

20. The plant of claim 13, wherein the polynucleotide is derived from the signal sequence of a golgi targeted gene.

21. The plant of claim 20, wherein the targeted gene is a rat sialyl transferease signal sequence.

22. The plant of claim 13, wherein the polynucleotide is derived from the signal sequence of an apoplast signal sequence.

23. The plant of claim 22, wherein the signal sequence is from *Aspergillus niger* ferulic acid esterase.

24. The plant of claim 16, wherein the polynucleotide is derived from *Solanum tuberosum.*

25. The plant of claim 13, wherein the polynucleotide sequence is downstream of the C-terminus of the ferulic acid esterase polynucleotide

26. The plant of claim 25, wherein the polynucleotide sequence is a KDEL sequence.

27. The plant of claim 25, wherein the polynucleotide sequence is a stop codon.

28. The plant of claim 25, wherein the polynucleotide sequence is an extension of the ferulic acid esterase reading frame to provide a linker to KDEL.

29. The plant of claim 1, further comprising introduction into the plant a second expression cassette comprising a promoter operably linked to a xylanase encoding polynucleotide.

30. The plant of claim 29, wherein the xylanase encoding polynucleotide is from *Trichoderma reesei*.

31. The plant of claim 29, wherein the first and second expression cassettes are present on separate plasmids.

32. The transgenic plant of claim 1, selected from the group consisting of Festuca, Lolium, Zea and Avena.

33. The transgenic plant of claim 32, wherein the plant is a Festuca plant.

34. A method of controlling the level of phenolic acids in plant cell walls of a transgenic plant, the method comprising introducing into the plant an expression cassette comprising a promoter operably linked to a ferulic acid esterase encoding polynucleotide.

35. The method of claim 34, wherein the polynucleotide is derived from *Aspergillus niger*.

36. The method of claim 35, wherein the polynucleotide is a FAE 1 gene from *Aspergillus niger*.

37. The method of claim 36, wherein the polynucleotide encodes the ferulic acid esterase with an altered glycosylation site.

38. The method of claim 36, wherein the polynucleotide encodes the ferulic acid esterase with a substitution such that glycosylation is altered.

39. The method of claim 36, wherein the polynucleotide

comprises CTWPVAAA at the 3' end.

40. The method of claim 36 wherein sub-optimal codons are modified to *Triticum spp.* preferred codons.

41. The method of claim 36, wherein the polynucleotide comprises SEQ ID NO:1.

42. The method of claim 34, wherein the introduction of the ferulic acid esterase polynucleotide into the plant is by transformation of cell cultures.

43. The method of claim 42, wherein the cell cultures are regenerated to plants.

44. The method of claim 34 wherein the ferulic acid esterase polynucleotide is introduced into the plant by sexual reproduction.

45. The method of claim 34, wherein the transgenic plant is a member of a genus selected from the group consisting of Festuca, Lolium, Avena and Zea.

46. The method of claim 45, wherein the transgenic plant is a member of the genus Festuca.

47. The method of claim 46, wherein the transgenic plant is a *Festuca arundinacea*.

48. The method of claim 34, wherein the promoter is an inducible promoter.

49. The method of claim 48, wherein the promoter is a senescence promoter.

50. The method of claim 48, wherein the promoter is a heat shock protein promoter.

51. The method of claim 34, wherein the promoter is a constitutive promoter.

52. The method of claim 51, wherein the promoter is an actin

promoter.

53. The method of claim 34, wherein the expression cassette further comprises a polynucleotide sequence that targets expression of the polynucleotide.

54. The method of claim 53, wherein the polynucleotide sequence is upstream of the N-terminus of the ferulic acid esterase polynucleotide.

55. The method of claim 54, wherein the polynucleotide is derived from the signal sequence of a vacuolar targeted gene.

56. The method of claim 55, wherein the targeted gene is a barley aleurain gene.

57. The method of claim 55, wherein the polynucleotide is derived from the signal sequence of a Lolium See1 signal sequence.

58. The method of claim 55, wherein the vacuolar signal sequence of the polynucleotide is modified to produce a endoplasmic reticulum signal sequence.

59. The method of claim 55, wherein the vacuolar signal sequence of the polynucleotide is modified to produce an apoplast signal sequence.

60. The method of claim 54, wherein the polynucleotide is derived from the signal sequence of a golgi targeted gene.

61. The method of claim 60, wherein the targeted gene is a rat sialyl transferease signal sequence.

62. The method of claim 59, wherein the polynucleotide is derived from the signal sequence of a fungal apoplast signal sequence.

63. The method of claim 62, wherein the signal sequence is from *Aspergillus niger* ferulic acid esterase.

64. The method of claim 59, wherein the apoplast signal

sequence is from a potato.

65. The method of claim 53, wherein the polynucleotide sequence is downstream of the C-terminus of the ferulic acid esterase polynucleotide

66. The method of claim 65, wherein the polynucleotide sequence is a KDEL sequence.

67. The method of claim 65, wherein the polynucleotide sequence is a stop codon.

68. The method of claim 65, wherein the polynucleotide sequence is an extension of the ferulic acid esterase reading frame to provide a linker to KDEL.

69. The method of claim 34, further comprising simultaneous introduction into the plant a second expression cassette comprising a promoter operably linked to a polynucleotide encoding a xylanase gene.

70. The method of claim 69, wherein the second polynucleotide is a fungal xylanase.

71. The method of claim 70, wherein the fungal xylanase is from *Trichoderma reesei.*

72. The method of claim 35, wherein the first and second expression cassettes are present on separate plasmids.

73. The method of claim 1, wherein the first and second expression cassettes are present on separate plasmids.

74. A transgenic plant produced by the method of claim 34.

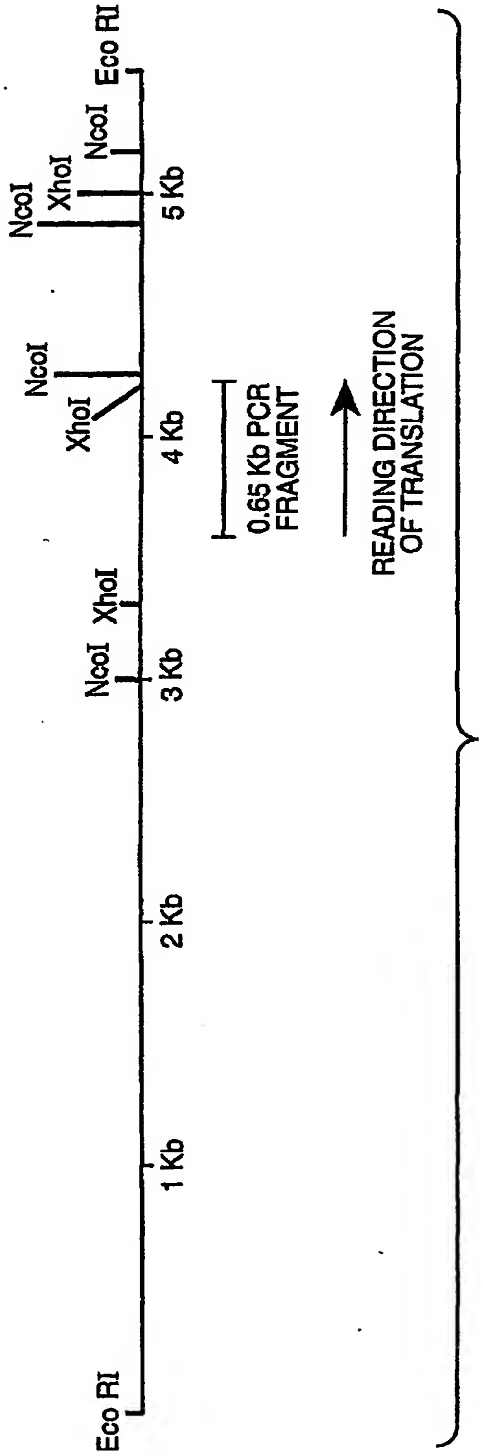
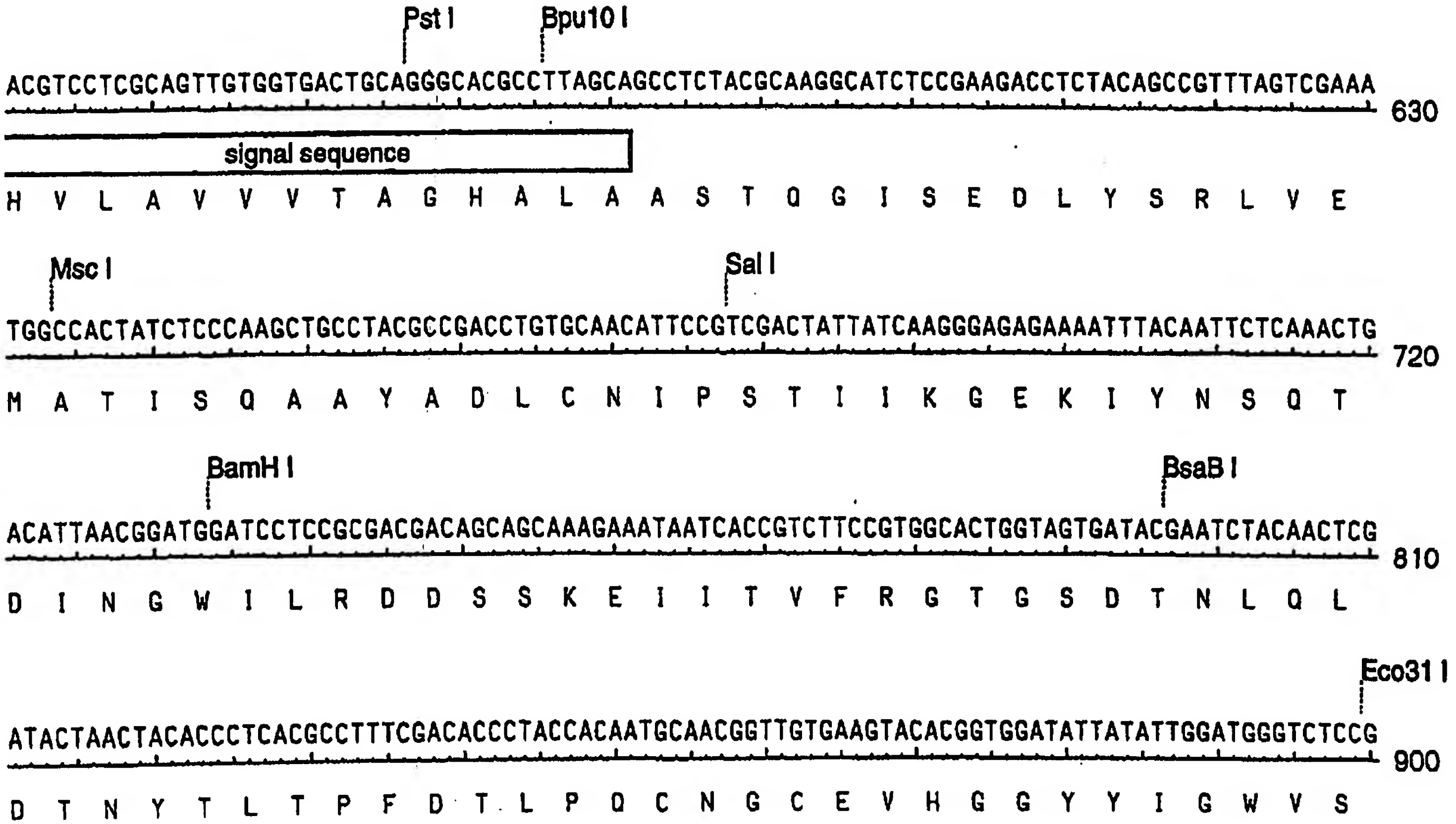

Eco RI
1 Kb
2 Kb
NcoI
3 Kb
XhoI
0.65 Kb PCR FRAGMENT
XhoI
4 Kb
NcoI
READING DIRECTION OF TRANSLATION
NcoI
5 Kb
XhoI
NcoI
Eco RI

FIG._1


Nco I
EcoR V
Psp1406 I
CCATGGTGGTGTCGATATCGGCAGTAGTCTTTGCCGAAACGTTGAGGGTTACAGTGATCTGCGTCGGACATACTTCGGGGAATCTACGGC
90
Sac I
GGAATATCAAAGTCTTCGGAATATCCATATTGGGAAAGGACAGAAGCTCCGGGGTAGTTTGATAGATGAGCTCCGGTGTATTAAATCGGG
180
BssH II
AGCTGACAGGAGTGAGCGTCATGTAGACCATCTAGTAATGTCAGTCGCGCGCAATTTCGCACATGAAACAAGTTGATTTCGGGACCCCAT
270
Xho I
Bst1107 I
EclHK I
TGTTACATCTCTCGGCTACAGCTCGAGATGTGCCTGCCGAGTATACTTAGAAGCCATGCCAGCGTGTTGTTATACGACCAAAAGTCAGGG
360
Pvu I
AATATGAAACGATCGTCGGATATTTCTTGTTTTTATCCTAAATTAGTCTTCCAGTGGTTTATTTAAGAGATAGATCCCTTCACAAACACT
450
Xmn I
CATCCAACGGACTTCTCATACCACTCATTGACATAATTTCAAACAGCTCCAGGCGCATTTAGTTCAACATGAAGCAATTCTCCGCCAAAC
540
signal sequence
M K Q F S A K

FIG._2A



ACGTCCTCGCAGTTGTGGTGACTGCAGGGCACGCCTTAGCAGCCTCTACGCAAGGCATCTCCGAAGACCTCTACAGCCGTTTAGTCGAAA 630

H V L A V V V T A G H A L A A S T Q G I S E D L Y S R L V E

TGGCCACTATCTCCCAAGCTGCCTACGCCGACCTGTGCAACATTCCGTCGACTATTATCAAGGGAGAGAAAATTTACAATTCTCAAACTG 720

M A T I S Q A A Y A D L C N I P S T I I K G E K I Y N S Q T

ACATTAACGGATGGATCCTCCGCGACGACAGCAGCAAAGAAATAATCACCGTCTTCCGTGGCACTGGTAGTGATACGAATCTACAACTCG 810

D I N G W I L R D D S S K E I I T V F R G T G S D T N L Q L

ATACTAACTACACCCTCACGCCTTTCGACACCCTACCACAATGCAACGGTTGTGAAGTACACGGTGGATATTATATTGGATGGGTCTCCG 900

D T N Y T L T P F D T L P Q C N G C E V H G G Y Y I G W V S

FIG._2B

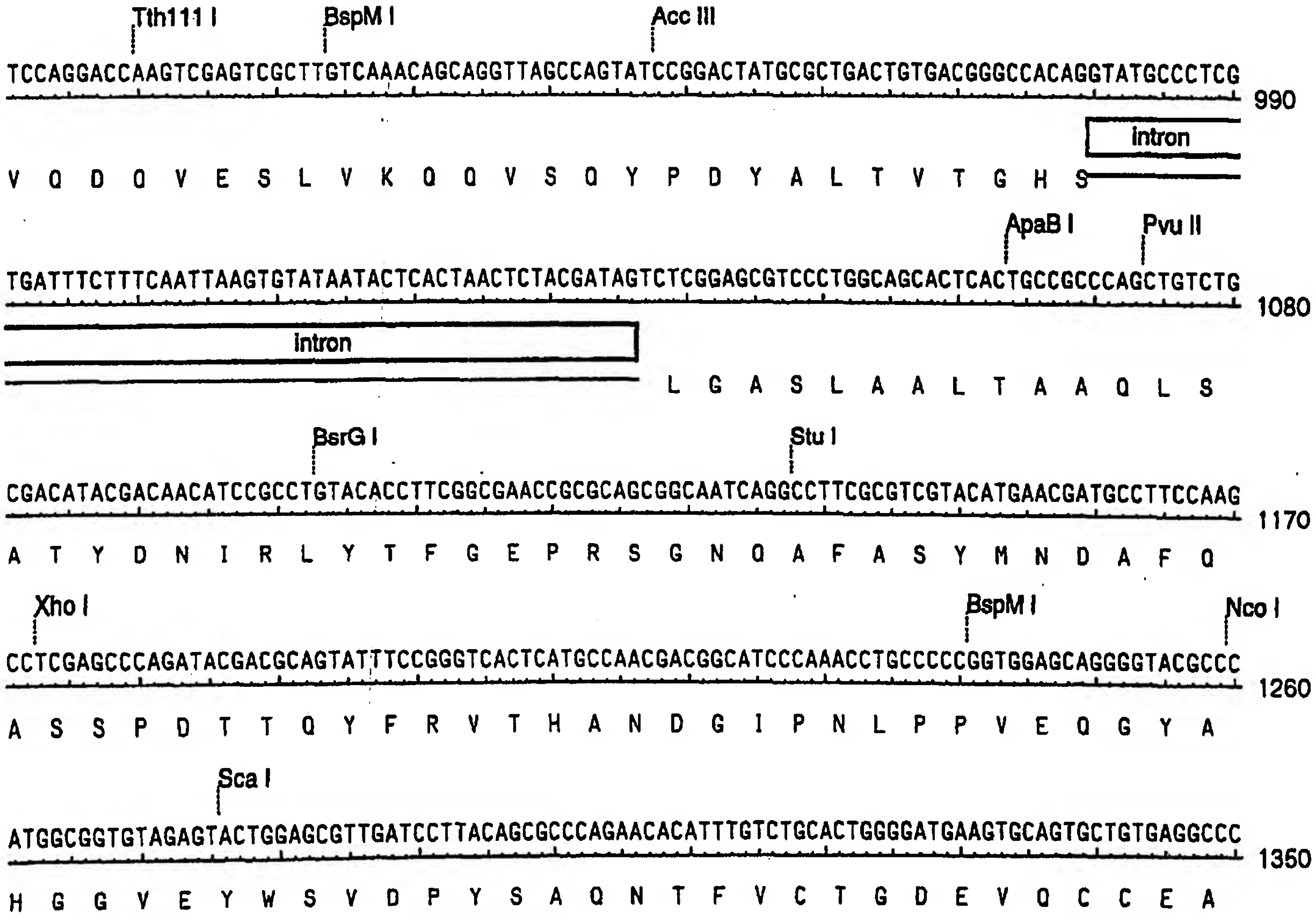

Tth111 I
BspM I
Acc III
TCCAGGACCAAGTCGAGTCGCTTGTCAAACAGCAGGTTAGCCAGTATCCGGACTATGCGCTGACTGTGACGGGCCACAGGTATGCCCTCG
990
intron
V Q D Q V E S L V K Q Q V S Q Y P D Y A L T V T G H S
ApaB I
Pvu II
TGATTTCTTTCAATTAAGTGTATAATACTCACTAACTCTACGATAGTCTCGGAGCGTCCCTGGCAGCACTCACTGCCGCCCAGCTGTCTG
1080
intron
L G A S L A A L T A A Q L S
BsrG I
Stu I
CGACATACGACAACATCCGCCTGTACACCTTCGGCGAACCGCGCAGCGGCAATCAGGCCTTCGCGTCGTACATGAACGATGCCTTCCAAG
1170
A T Y D N I R L Y T F G E P R S G N Q A F A S Y M N D A F Q
Xho I
BspM I
Nco I
CCTCGAGCCCAGATACGACGCAGTATTTCCGGGTCACTCATGCCAACGACGGCATCCCAAACCTGCCCCCGGTGGAGCAGGGGTACGCCC
1260
A S S P D T T Q Y F R V T H A N D G I P N L P P V E Q G Y A
Sca I
ATGGCGGTGTAGAGTACTGGAGCGTTGATCCTTACAGCGCCCAGAACACATTTGTCTGCACTGGGGATGAAGTGCAGTGCTGTGAGGCCC
1350
H G G V E Y W S V D P Y S A Q N T F V C T G D E V Q C C E A

FIG._2C

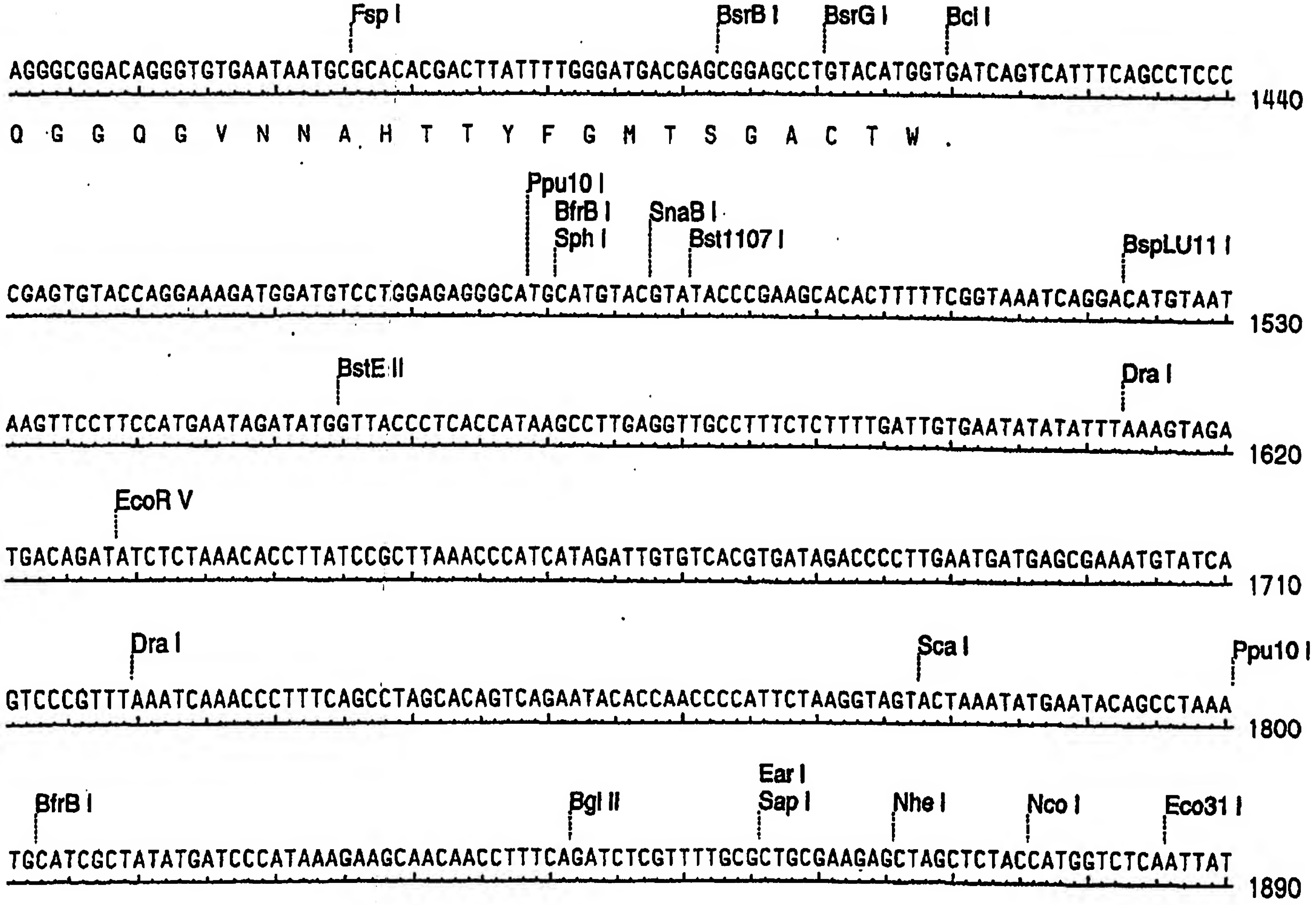

Fsp I
BsrB I
BsrG I
Bcl I
AGGGCGGACAGGGTGTGAATAATGCGCACACGACTTATTTTGGGATGACGAGCGGAGCCTGTACATGGTGATCAGTCATTTCAGCCTCCC
1440
Q G G Q G V N N A H T T Y F G M T S G A C T W .
Ppu10 I
BfrB I
Sph I
SnaB I
Bst1107 I
BspLU11 I
CGAGTGTACCAGGAAAGATGGATGTCCTGGAGAGGGCATGCATGTACGTATACCCGAAGCACACTTTTTCGGTAAATCAGGACATGTAAT
1530
BstE II
Dra I
AAGTTCCTTCCATGAATAGATATGGTTACCCTCACCATAAGCCTTGAGGTTGCCTTTCTCTTTTGATTGTGAATATATATTTAAAGTAGA
1620
EcoR V
TGACAGATATCTCTAAACACCTTATCCGCTTAAACCCATCATAGATTGTGTCACGTGATAGACCCCTTGAATGATGAGCGAAATGTATCA
1710
Dra I
Sca I
Ppu10 I
GTCCCGTTTAAATCAAACCCTTTCAGCCTAGCACAGTCAGAATACACCAACCCCATTCTAAGGTAGTACTAAATATGAATACAGCCTAAA
1800
BfrB I
Bgl II
Ear I
Sap I
Nhe I
Nco I
Eco31 I
TGCATCGCTATATGATCCCATAAAGAAGCAACAACCTTTCAGATCTCGTTTTGCGCTGCGAAGAGCTAGCTCTACCATGGTCTCAATTAT
1890

FIG._2D

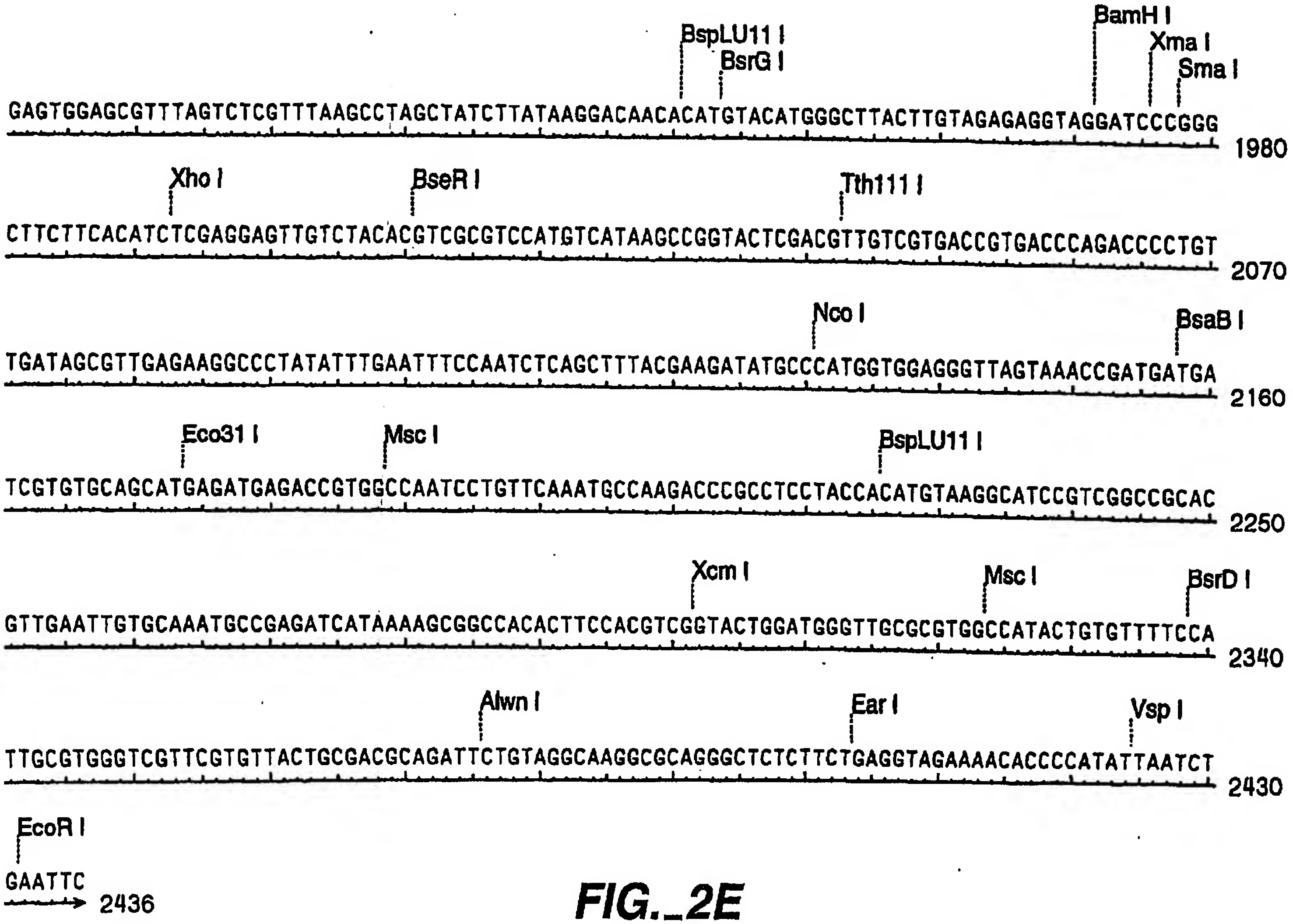

BspLU11 I
BsrG I
BamH I
Xma I
Sma I
GAGTGGAGCGTTTAGTCTCGTTTAAGCCTAGCTATCTTATAAGGACAACACATGTACATGGGCTTACTTGTAGAGAGGTAGGATCCCGGG
1980
Xho I
BseR I
Tth111 I
CTTCTTCACATCTCGAGGAGTTGTCTACACGTCGCGTCCATGTCATAAGCCGGTACTCGACGTTGTCGTGACCGTGACCCAGACCCCTGT
2070
Nco I
BsaB I
TGATAGCGTTGAGAAGGCCCTATATTTGAATTTCCAATCTCAGCTTTACGAAGATATGCCCATGGTGGAGGGTTAGTAAACCGATGATGA
2160
Eco31 I
Msc I
BspLU11 I
TCGTGTGCAGCATGAGATGAGACCGTGGCCAATCCTGTTCAAATGCCAAGACCCGCCTCCTACCACATGTAAGGCATCCGTCGGCCGCAC
2250
Xcm I
Msc I
BsrD I
GTTGAATTGTGCAAATGCCGAGATCATAAAAGCGGCCACACTTCCACGTCGGTACTGGATGGGTTGCGCGTGGCCATACTGTGTTTTCCA
2340
Alwn I
Ear I
Vsp I
TTGCGTGGGTCGTTCGTGTTACTGCGACGCAGATTCTGTAGGCAAGGCGCAGGGCTCTCTTCTGAGGTAGAAAACACCCCATATTAATCT
2430
EcoR I
GAATTC
2436

FIG._2E

```
CCATGGTGGTGTCGATATCGGCAGTAGTCTTTGCCGAAACGTTGAGGGTTACAGTGATCTGCGTCGGACATACTT
CGGGGAATCTACGGCGGAATATCAAAGTCTTCGGAATATCCATATTGGGAAAGGACAGAAGCTCCGGGGTAGTTT
GATAGATGAGCTCCGGTGTATTAAATCGGGAGCTGACAGGAGTGAGCGTCATGTAGACCATCTAGTAATGTCAGT
CGCGCGCAATTTCGCACATGAAACAAGTTGATTTCGGGACCCCATTGTTACATCTCTCGGCTACAGCTCGAGATG
TGCCTGCCGAGTATACTTAGAAGCCATGCCAGCGTGTTGTTATACGACCAAAAGTCAGGGAATATGAAACGATCG
TCGGATATTTCTTGTTTTTATCCTAAATTAGTCTTCCAGTGGTTTATTTAAGAGATAGATCCCTTCACAAACACT
CATCCAACGGACTTCTCATACCACTCATTGACATAATTTCAAACAGCTCCAGGCGCATTTAGTTCAACATGAAGC
AATTCTCCGCCAAACACGTCCTCGCAGTTGTGGTGACTGCAGGGCACGCCTTAGCAGCCTCTACGCAAGGCATCT
CCGAAGACCTCTACAGCCGTTTAGTCGAAATGGCCACTATCTCCCAAGCTGCCTACGCCGACCTGTGCAACATTC
CGTCGACTATTATCAAGGGAGAGAAAATTTACAATTCTCAAACTGACATTAACGGATGGATCCTCCGCGACGACA
GCAGCAAAGAAATAATCACCGTCTTCCGTGGCACTGGTAGTGATACGAATCTACAACTCGATACTAACTACACCC
TCACGCCTTTCGACACCCTACCACAATGCAACGGTTGTGAAGTACACGGTGGATATTATATTGGATGGGTCTCCG
TCCAGGACCAAGTCGAGTCGCTTGTCAAACAGCAGGTTAGCCAGTATCCGGACTATGCGCTGACTGTGACGGGCC
ACAGGTATGCCCTCGTGATTTCTTTCAATTAAGTGTATAATACTCACTAACTCTACGATAGTCTCGGAGCGTCCC
TGGCAGCACTCACTGCCGCCCAGCTGTCTGCGACATACGACAACATCCGCCTGTACACCTTCGGCGAACCGCGCA
GCGGCAATCAGGCCTTCGCGTCGTACATGAACGATGCCTTCCAAGCCTCGAGCCCAGATACGACGCAGTATTTCC
GGGTCACTCATGCCAACGACGGCATCCCAAACCTGCCCCCGGTGGAGCAGGGGTACGCCCATGGCGGTGTAGAGT
ACTGGAGCGTTGATCCTTACAGCGCCCAGAACACATTTGTCTGCACTGGGGATGAAGTGCAGTGCTGTGAGGCCC
AGGGCGGACAGGGTGTGAATAATGCGCACACGACTTATTTTGGGATGACGAGCGGAGCCTGTACATGGTGATCAG
TCATTTCAGCCTCCCCGAGTGTACCAGGAAAGATGGATGTCCTGGAGAGGGCATGCATGTACGTATACCCGAAGC
ACACTTTTTCGGTAAATCAGGACATGTAATAAGTTCCTTCCATGAATAGATATGGTTACCCTCACCATAAGCCTT
GAGGTTGCCTTTCTCTTTTGATTGTGAATATATATTTAAAGTAGATGACAGATATCTCTAAACACCTTATCCGCT
TAAACCCATCATAGATTGTGTCACGTGATAGACCCCTTGAATGATGAGCGAAATGTATCAGTCCCGTTTAAATCA
AACCCTTTCAGCCTAGCACAGTCAGAATACACCAACCCCATTCTAAGGTAGTACTAAATATGAATACAGCCTAAA
TGCATCGCTATATGATCCCATAAAGAAGCAACAACCTTTCAGATCTCGTTTTGCGCTGCGAAGAGCTAGCTCTAC
CATGGTCTCAATTATGAGTGGAGCGTTTAGTCTCGTTTAAGCCTAGCTATCTTATAAGGACAACACATGTACATG
GGCTTACTTGTAGAGAGGTAGGATCCCGGGCTTCTTCACATCTCGAGGAGTTGTCTACACGTCGCGTCCATGTCA
TAAGCCGGTACTCGACGTTGTCGTGACCGTGACCCAGACCCCTGTTGATAGCGTTGAGAAGGCCCTATATTTGAA
TTTCCAATCTCAGCTTTACGAAGATATGCCCATGGTGGAGGGTTAGTAAACCGATGATGATCGTGTGCAGCATGA
GATGAGACCGTGGCCAATCCTGTTCAAATGCCAAGACCCGCCTCCTACCACATGTAAGGCATCCGTCGGCCGCAC
GTTGAATTGTGCAAATGCCGAGATCATAAAAGCGGCCACACTTCCACGTCGGTACTGGATGGGTTGCGCGTGGCC
ATACTGTGTTTTCCATTGCGTGGGTCGTTCGTGTTACTGCGACGCAGATTCTGTAGGCAAGGCGCAGGGCTCTCT
TCTGAGGTAGAAAACACCCCATATTAATCTGAATTC
```

FIG._3

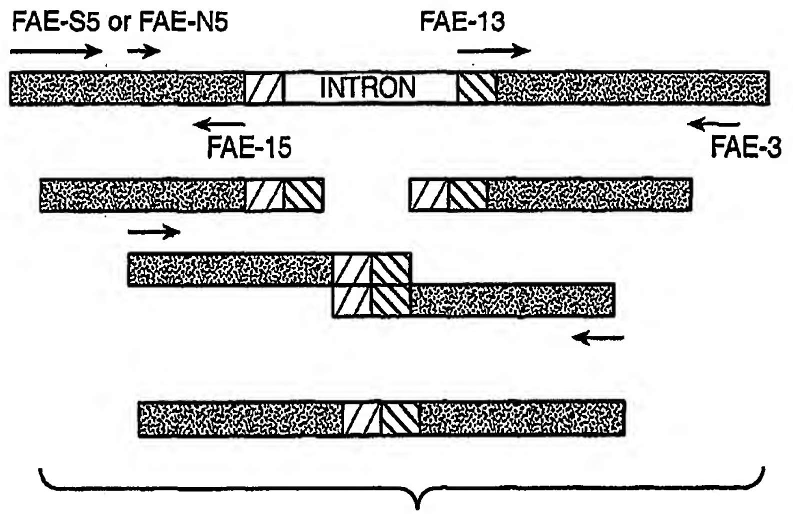


FIG._4

```
FAE-I3 CCGGCCACGCCCTCGGCGCCTCCCTGGCGGCACTC     35-mer
FAE-I5 GGCGCCGAGGGAGTGGCCGGTCACGGTCAGCGCGTAGTCC        40-mer

                              i                        intron position in original

    Y  A  L  T  V  T  G  H  S   L  G  A  S  L  A  A  L
GGACTACGCGCTGACCGTGACCGGCCACTCCCTCGGCGCC                       complement, FAE-I5
                    CCGGCCACGCCCTCGGCGCCTCCCTGGCGGCACTC                    FAE-I3
    Y  A  L  T  V  T  G  H  A   L  G  A  S  L  A  A  L
```

FIG._5

Vector Construction

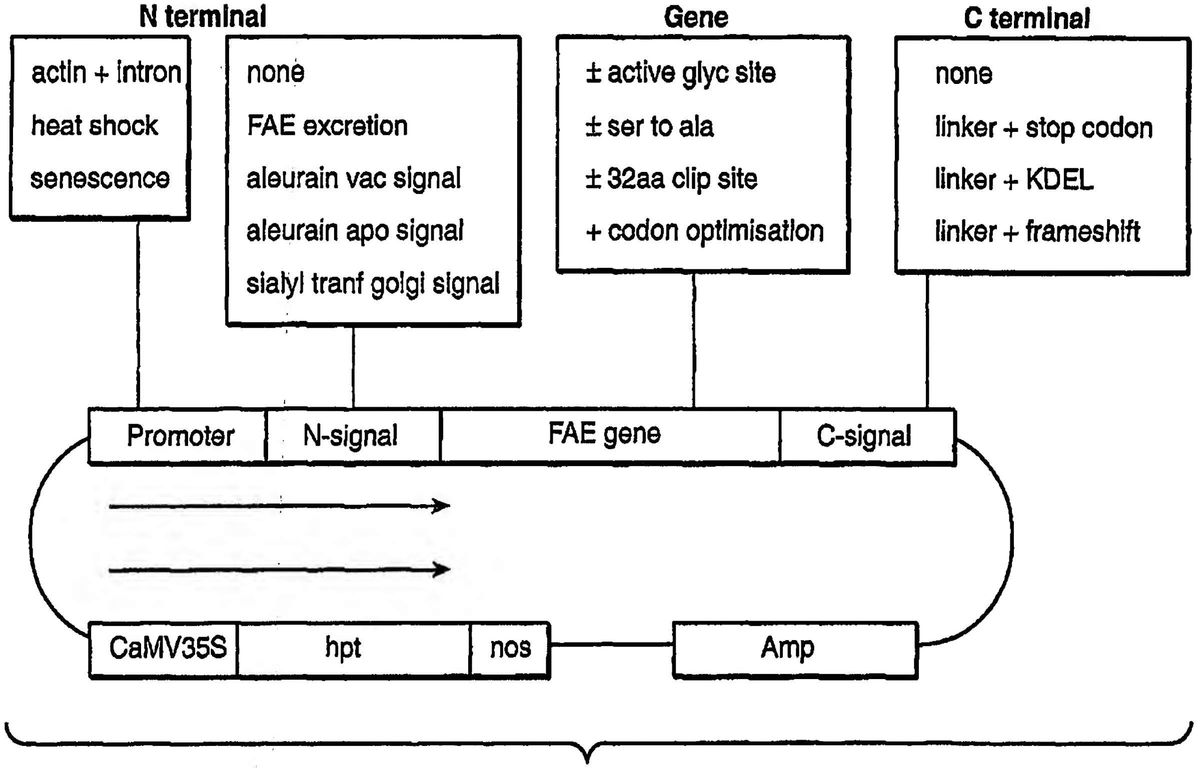


FIG._6

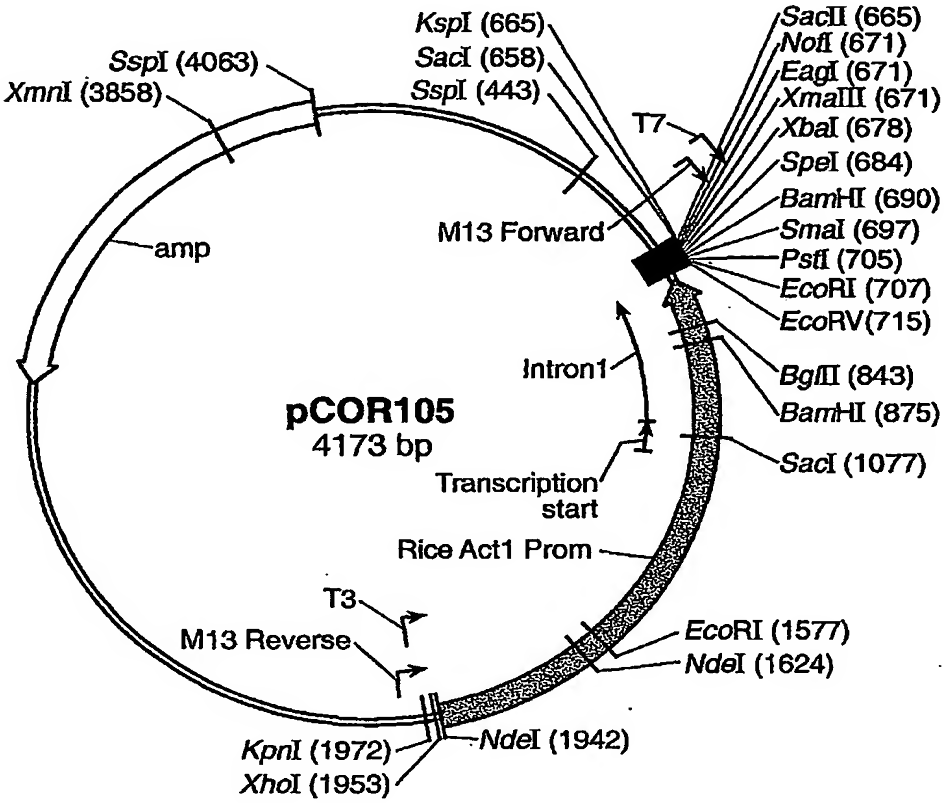

KspI (665)
SacI (658)
SspI (443)
SspI (4063)
XmnI (3858)
SacII (665)
NotI (671)
EagI (671)
XmaIII (671)
XbaI (678)
SpeI (684)
BamHI (690)
SmaI (697)
PstI (705)
EcoRI (707)
EcoRV (715)
BglII (843)
BamHI (875)
SacI (1077)
T7
M13 Forward
amp
Intron1
pCOR105
4173 bp
Transcription start
Rice Act1 Prom
T3
M13 Reverse
EcoRI (1577)
NdeI (1624)
NdeI (1942)
KpnI (1972)
XhoI (1953)

FIG._7

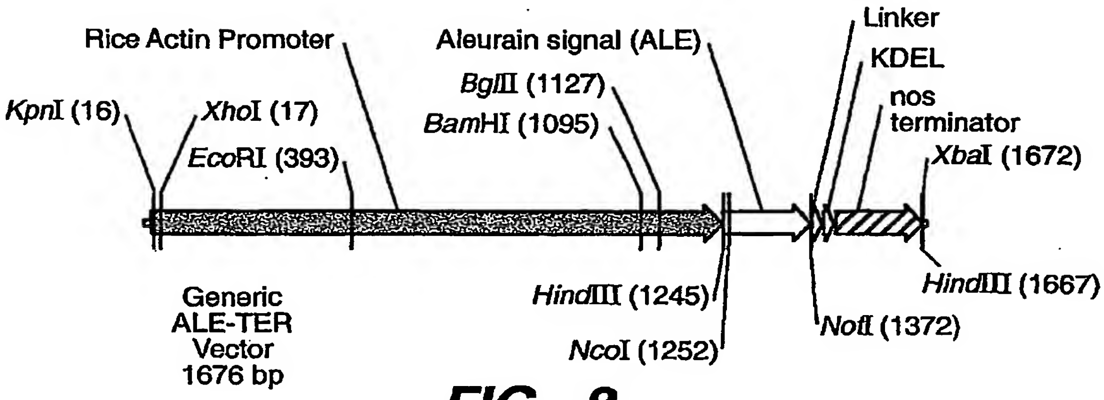


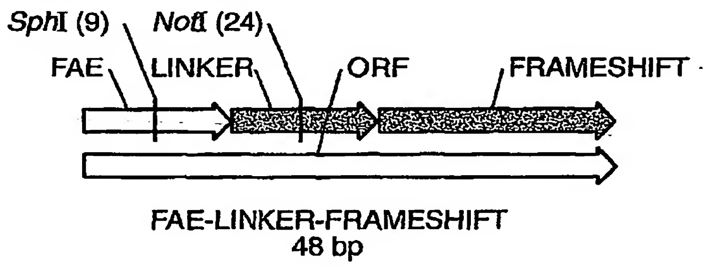
FIG._8

KDEL-COOH ER retention sequence

```
       NotI
     ~~~~~~~~
      A  A  A  K   P  L  K   D  E  L   *
1    GCGGCCGCGA AACCACTGAA GGATGAGCTG TAA
```

FIG._9

FAE-LINKER-FRAMESHIFT Structure and Sequence



```
+1  G   A   C   T   W   P   V   A   A   A   E   T   T   E   G
       SphI                       NotI
     ~~~~~~                     ~~~~~~~~
1   GGCGCATGCA CCTGGCCGGT CGCGGCCGCG GAAACCACTG AAGGATGA
    CCGCGTACGT GGACCGGCCA GCGCCGGCGC CTTTGGTGAC TTCCTACT
```

FIG._10

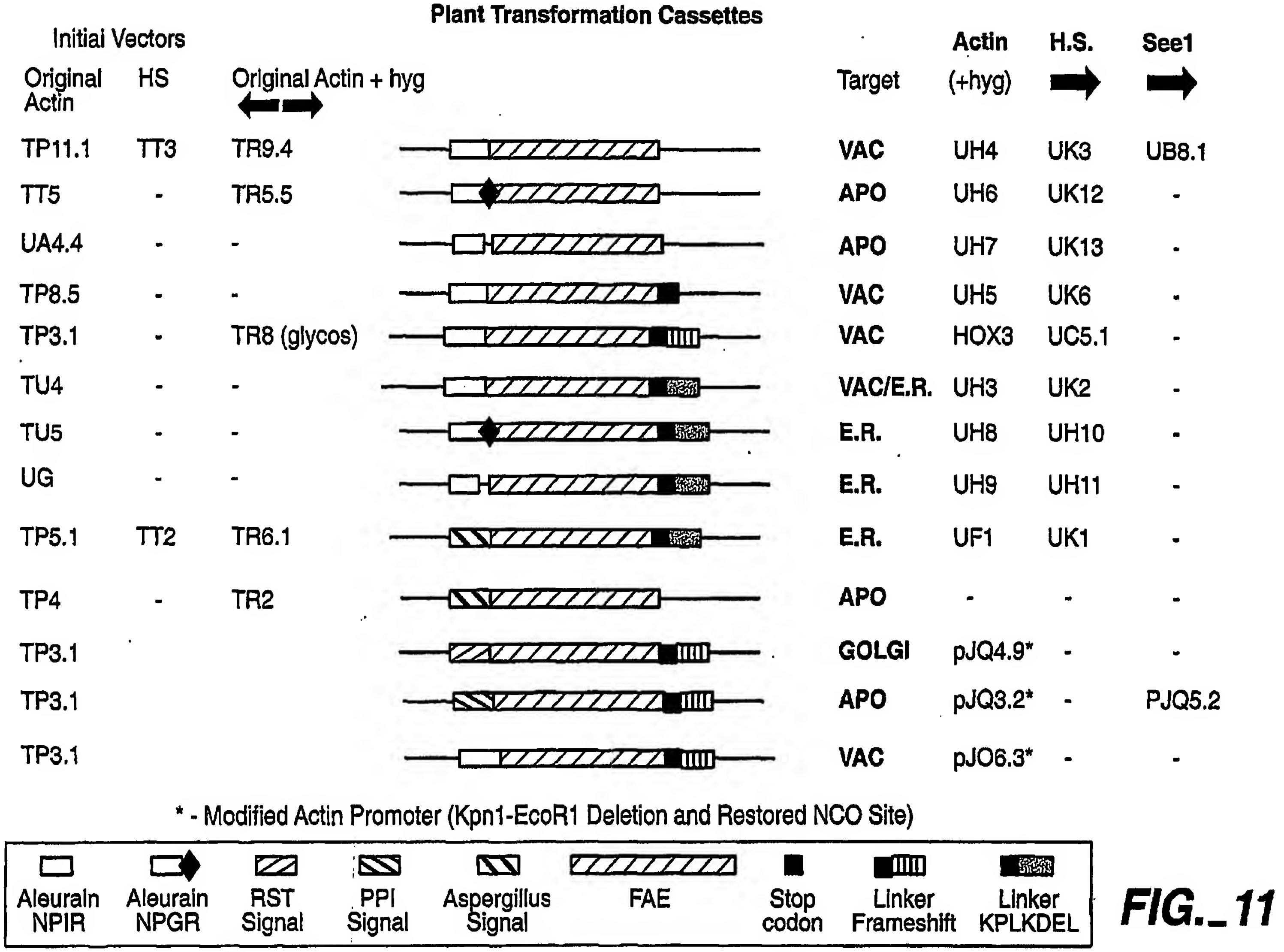

Plant Transformation Cassettes
Initial Vectors
Original Actin
HS
Original Actin + hyg
Target
Actin (+hyg)
H.S.
See1
TP11.1 TT3 TR9.4 VAC UH4 UK3 UB8.1
TT5 - TR5.5 APO UH6 UK12 -
UA4.4 - - APO UH7 UK13 -
TP8.5 - - VAC UH5 UK6 -
TP3.1 - TR8 (glycos) VAC HOX3 UC5.1 -
TU4 - - VAC/E.R. UH3 UK2 -
TU5 - - E.R. UH8 UH10 -
UG - - E.R. UH9 UH11 -
TP5.1 TT2 TR6.1 E.R. UF1 UK1 -
TP4 - TR2 APO - - -
TP3.1 GOLGI pJQ4.9* - -
TP3.1 APO pJQ3.2* - PJQ5.2
TP3.1 VAC pJO6.3* - -
* - Modified Actin Promoter (Kpn1-EcoR1 Deletion and Restored NCO Site)
Aleurain NPIR
Aleurain NPGR
RST Signal
PPI Signal
Aspergillus Signal
FAE
Stop codon
Linker Frameshift
Linker KPLKDEL

FIG._11

Vectors

Original Actin promoter in pCOR105

	Target	Signal sequences	Vectors
(i)	APO	- aleurain-NPGR-FAE	pUH6, pTT5, TT5.5, pTT5.1
		- aleurain-delNPIR -FAE	pUH7, pUA4.4,
(ii)	ER	- aleurain-NPGR-FAE-linker-KDEL	pTU5, pUH8,
		- aleurain-delNPIR-FAE-linker-KDEL	pUG4, pUH9,
(iii)	VAC	- aleurain-NPIR-FAE	pTP11.1, pTR9.4, pUH4, pUK3,
(iv)	ER/VAC	- aleurain-NPIR-FAE-linker-KDEL	pTU4, pUH3,
(v)	VAC	- aleurain-NPIR-FAE-linker-frameshift	pUA1K3, pTP3.1, pUC5.11
(vi)	VAC	- aleurain-NPIR-FAE-linker-stop	pTP8.5, pUH5
(vii)	ER	- Aspergillus signal -FAE-KDEL	pTP5.1, pTP6.1, pUF1,

Modified actin promoter (Kpn1-EcoR1 deletion and restored NCO site)

(i)	VAC	- aleurain-NPIR-FAE-linker-frameshift	pJ06.3
(ii)	GOLGI	- RST-FAE-linker-frameshift	pJQ3.2
(iii)	APO	- PPI-FAE-linker-frameshift	pJQ4.9

Heat-shock promoter

(i)	APO	- aleurain-NPGR-FAE	pUH12
		- aleurain-delNPIR-FAE	pUH13
		- Aspergillus signal-FAE	pTP4a2, pTR2.22,
(ii)	ER	- aleurain-NPGR-FAE-linker-KDEL	pUH10
		- aleurain-delNPIR-FAE-linker-KDEL	pUH11
(iii)	VAC	- aleurain-NPIR-FAE	pUK3,pTT3
(iv)	ER/VAC	- aleurain-NPIR-FAE-linker-KDEL	pUK2
(v)	VAC	- aleurain-NPIR-FAE-linker-frameshift	pUC5.11, pHOX3
(vi)	VAC	- aleurain-NPIR-FAE-linker-stop	pUK6
(vii)	ER	- Aspergillus signal -FAE-KDEL	pUK1, pTT2

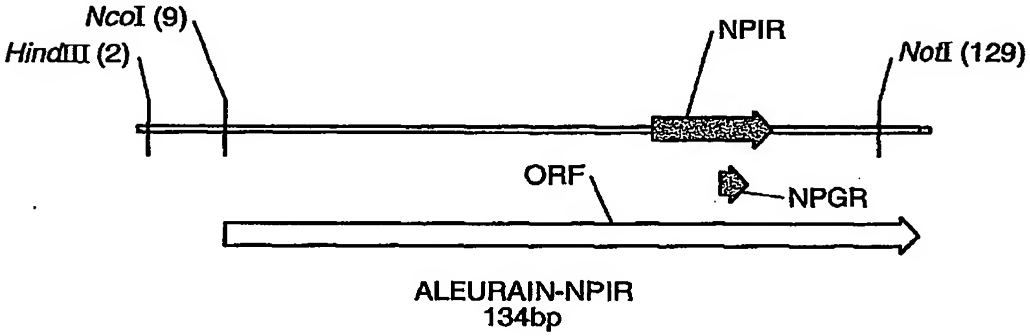
Senescence promoter

(i)	APO	- See1-PPI-FAE-linker-frameshift	pJQ5.2
(ii)	VAC	- See1-aleurain-deleted NPIR-FAE	pUB8.1

FIG._12

**ALEURAIN-NPIR (Vacuolar) and NPGR (Apoplast)
Structure and Sequence**

NPIR Underline
NPGR Bold



```
+1          M   A  H  A   R  V  L   L  L  A  L   A  V  L   A  T  A   A  V  A
   HindIII NcoI
   ~~~~~~ ~~~~~~~
1  AAGCTTACCA TGGCCCACGC CCGCGTCCTC CTCCTGGCGC TCGCCGTGCT GGCCACGGCC GCCGTCGCCG
   TTCGAATGGT ACCGGGTGCG GGCGCAGGAG GAGGACCGCG AGCGGCACGA CCGGTGCCGG CGGCAGCGGC

+1 V  A  S  S   S  S  F   A  D  S  N  P  I  R   P  V  T   D  R  A  A
                                                              NotI
                                                            ~~~~~~~~
71 TCGCCTCCTC CTCCTCCTTC GCCGACTCCA ACCCGATCCG GCCCGTCACC GACCGCGCGG CCGC
   AGCGGAGGAG GAGGAGGAAG CGGCTGAGGT TGGGCTAGGC CGGGCAGTGG CTGGCGCGCC GGCG
```

FIG._13

RAT SIALYL TRANSFERASE **Golgi signal sequence**

```
      HindIII
      ~~~~~~
                 M  I  H  T   N  L  K   K  K  F  S   L  F  I   L  V  F   L  L  F  A
  1   AAGCTTACCA TGATCCACAC CAACCTCAAA AAGAAGTTCT CCCTCTTCAT CCTCGTCTTC CTCCTCTTCG

      · V  I  C   V  W  K   K  G  S  D   Y  E  A   L  T  L   Q  A  K  E   F  Q  M
 71   CCGTGATCTG CGTGTGGAAG AAGGGCTCCG ACTACGAGGC CCTCACCCTC CAAGCCAAGG AGTTCCAAAT

        NotI
        ~~~~~~~~
      · A  A
141   GGCGGCCGC
```

FIG._14

POTATO PROTEASE INHIBITOR II **Apoplast signal sequence**

```
      HindIII
      ~~~~~~
                 M. X  V  H   K  E  V   N  F  V  A   Y  L  L   I  V  L   G  L  L  L
  1   AAGCTTACMA TGGMCGTGCA CAAGGAGGTS AACTTCGTSG CCTACCTCCT GATCGTSCTC
      GGCCTCCTCT
                    NcoI
                   ~~~~~~
      ·  L  V  S   A  M  E   H  V  D  A   K  A  C   T  X  E   C  G  N  L
      G  F  G ·
 71   TGCTCGTSTC CGCCATGGAG CACGTGGACG CCAAGGCCTG CACCCKCGAG TGCGGCAACC
      TCGGCTTCGG
                NotI
               ~~~~~~~~
      · I  C  P   A  A  A
141   CATCTGCCCG GCGGCCGCC
```

FIG._15

Targeting Expression of gfp to Different Cell Compartments

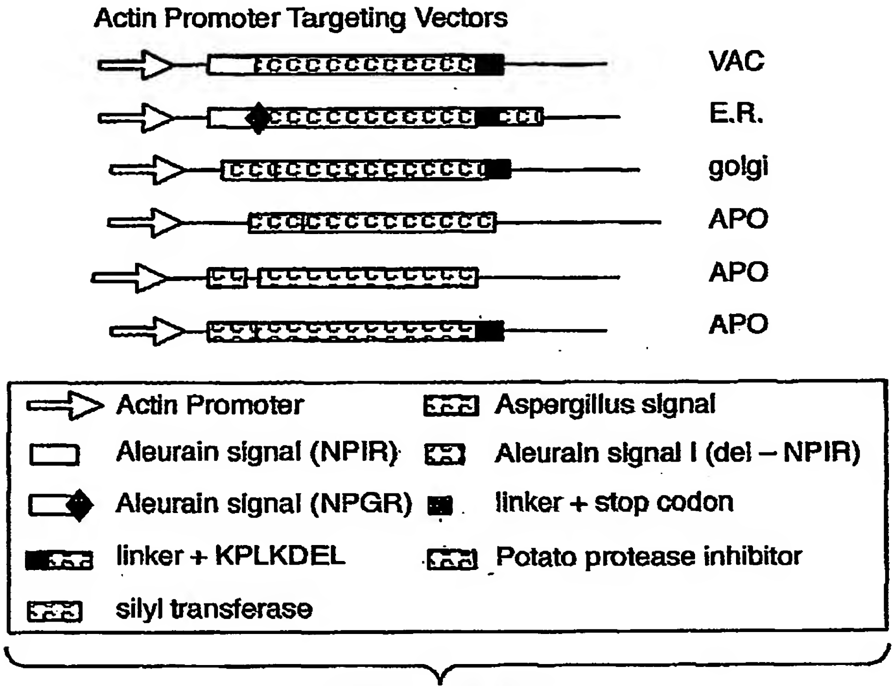


FIG._16A

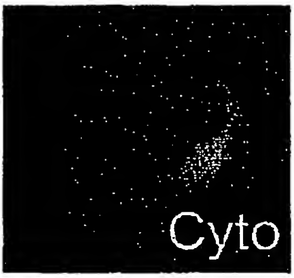


FIG._16B

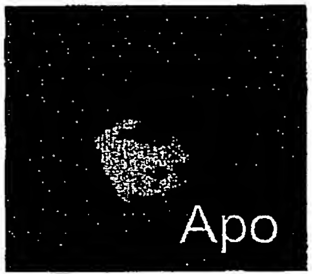


FIG._16C

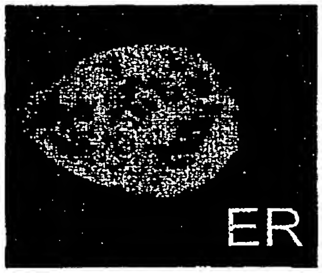


FIG._16D

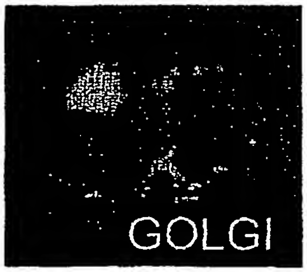


FIG._16E

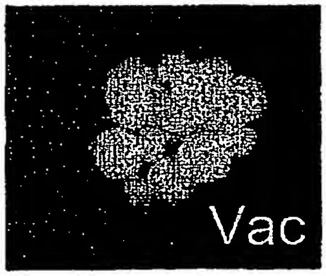


FIG._16F

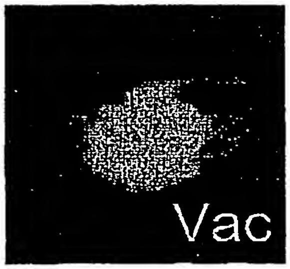


FIG._16G

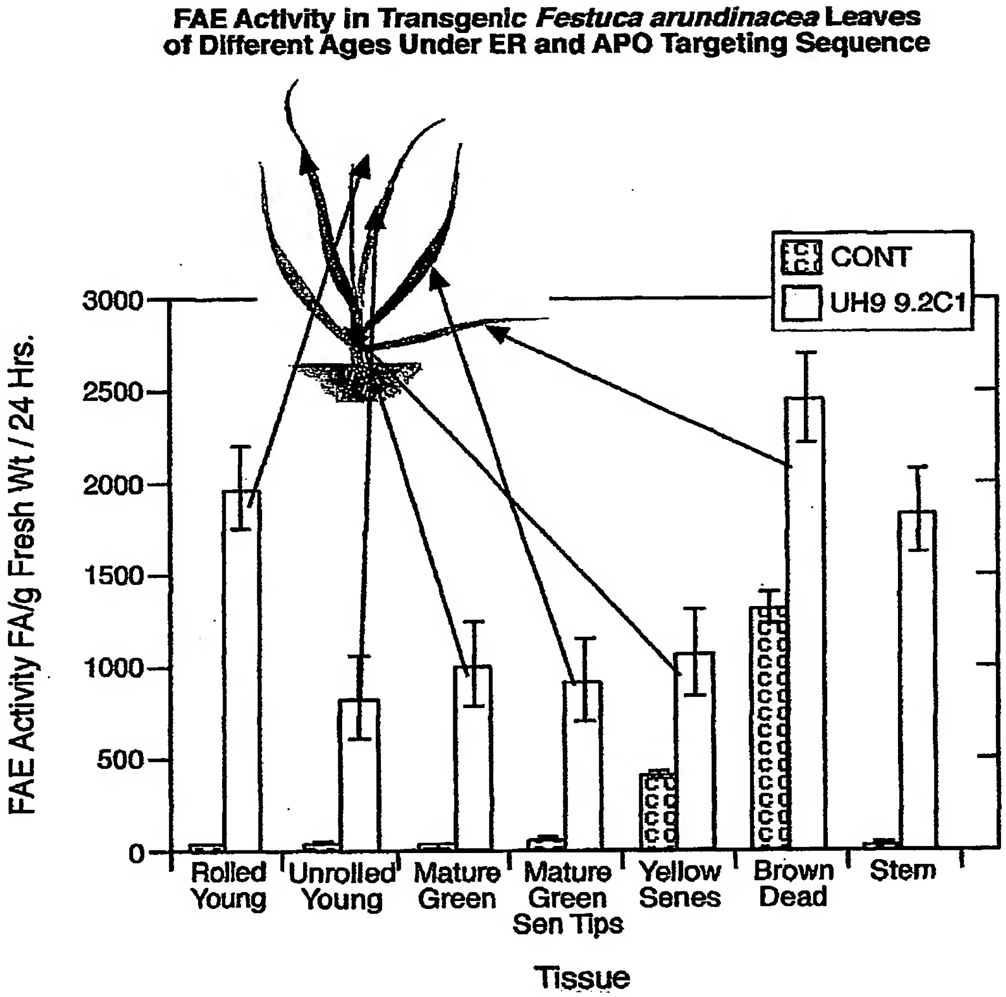

FAE Activity in Transgenic *Festuca arundinacea* Leaves
of Different Ages Under ER and APO Targeting Sequence
CONT
UH9 9.2C1
FAE Activity FA/g Fresh Wt / 24 Hrs.
3000
2500
2000
1500
1000
500
0
Rolled Young
Unrolled Young
Mature Green
Mature Green Sen Tips
Yellow Senes
Brown Dead
Stem
Tissue

FIG._17A

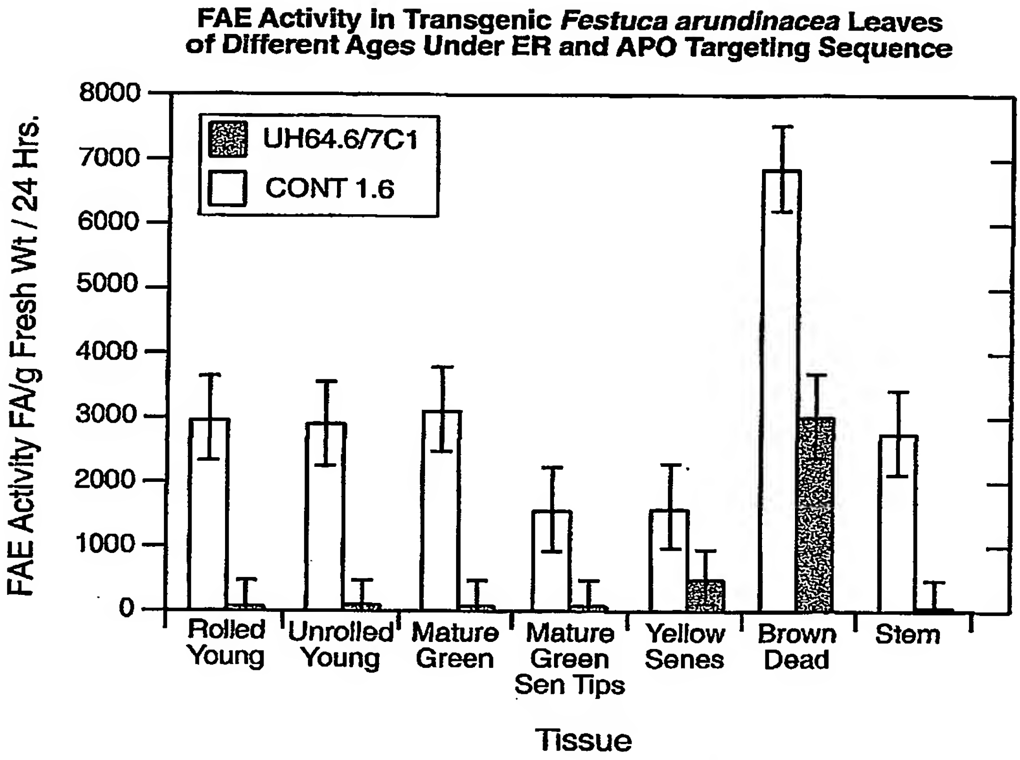

FAE Activity in Transgenic *Festuca arundinacea* Leaves of Different Ages Under ER and APO Targeting Sequence
FAE Activity FA/g Fresh Wt / 24 Hrs.
8000
7000
6000
5000
4000
3000
2000
1000
0
UH64.6/7C1
CONT 1.6
Rolled Young
Unrolled Young
Mature Green
Mature Green Sen Tips
Yellow Senes
Brown Dead
Stem
Tissue

FIG._17B

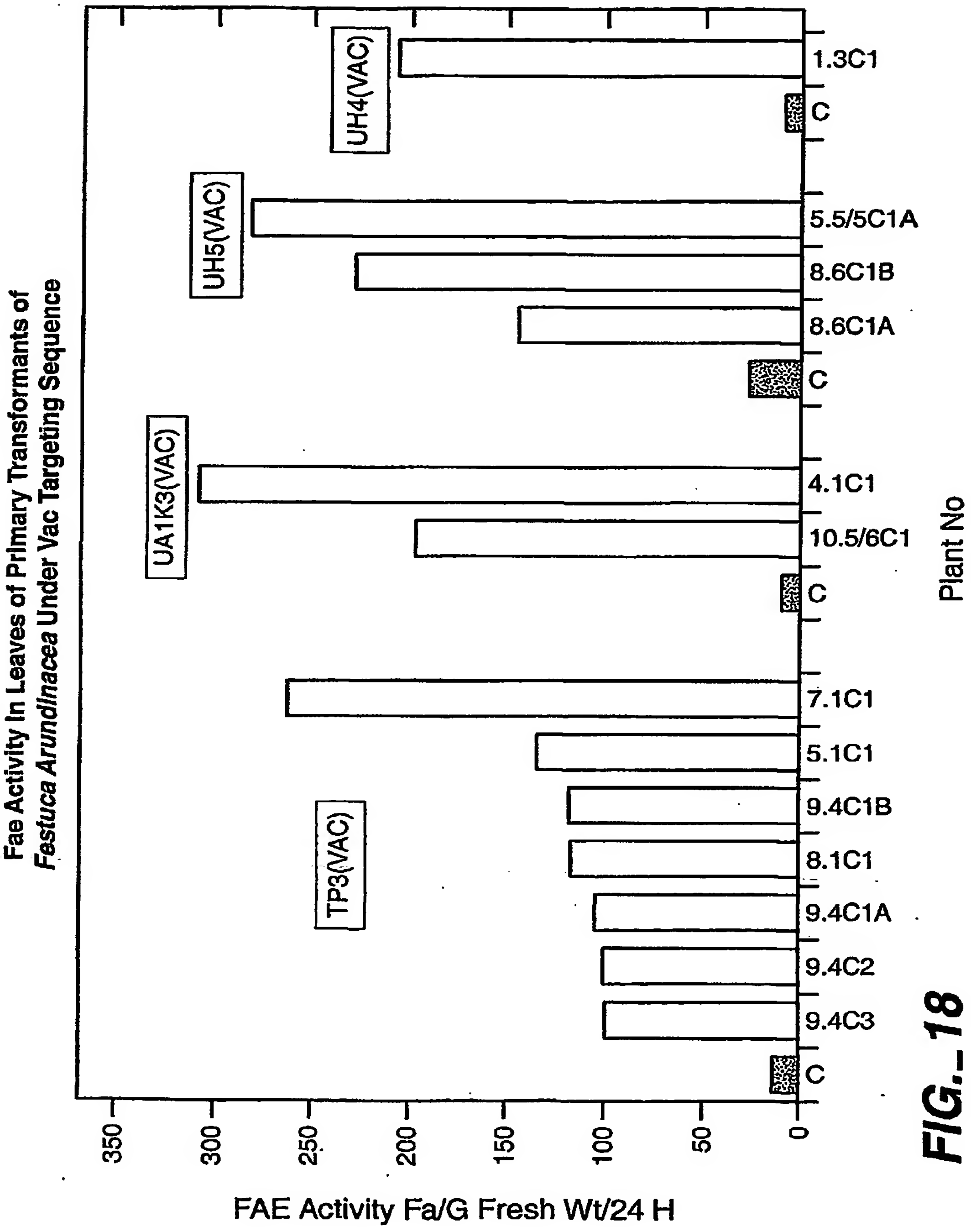
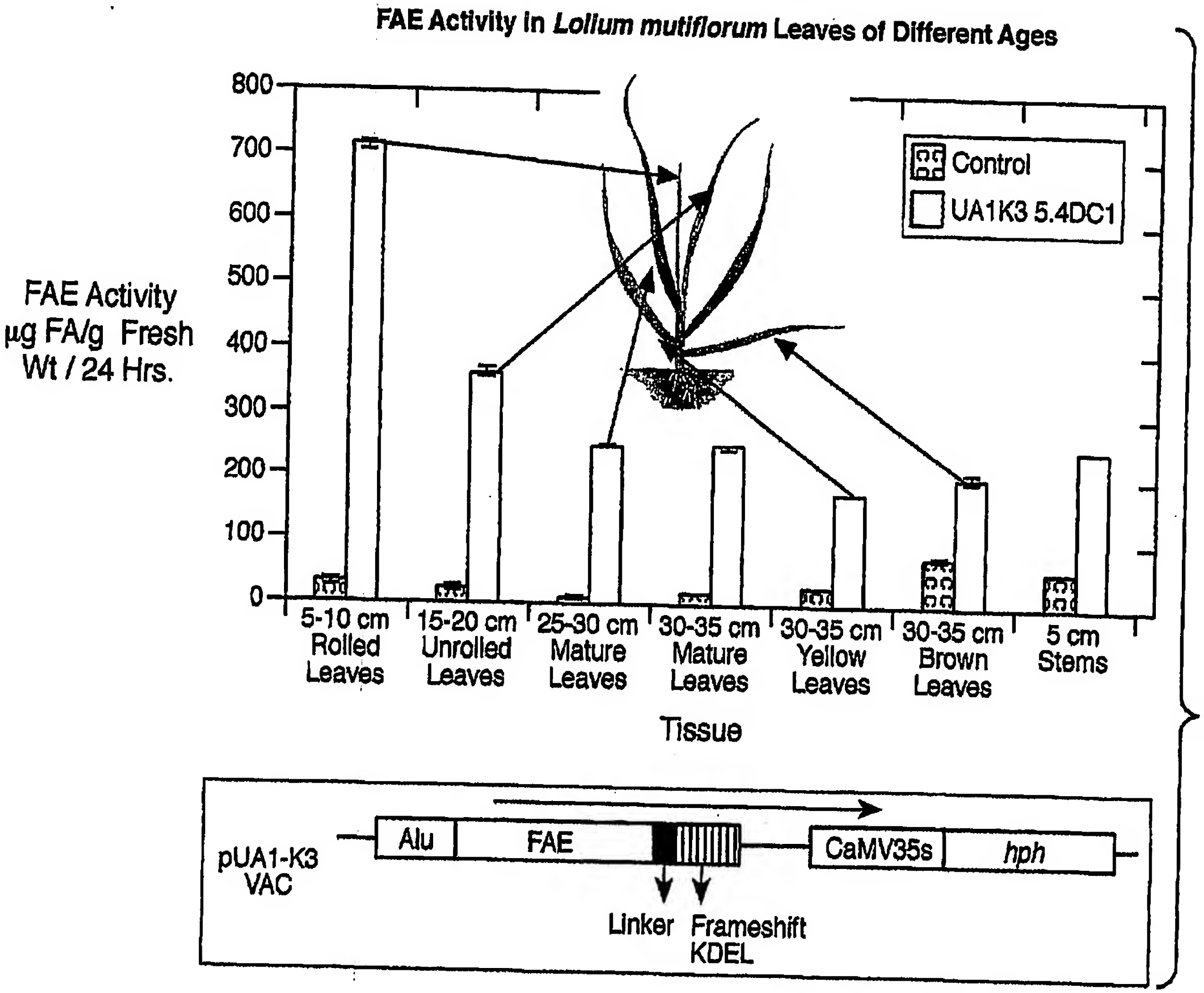

Fae Activity In Leaves of Primary Transformants of
Festuca Arundinacea Under Vac Targeting Sequence
UH4(VAC)
UH5(VAC)
UA1K3(VAC)
TP3(VAC)
1.3C1
C
5.5/5C1A
8.6C1B
8.6C1A
C
4.1C1
10.5/6C1
C
7.1C1
5.1C1
9.4C1B
8.1C1
9.4C1A
9.4C2
9.4C3
C
Plant No
350
300
250
200
150
100
50
0
FAE Activity Fa/G Fresh Wt/24 H

FIG._18



FAE Activity in *Lolium mutiflorum* Leaves of Different Ages

FIG._19

FAE Activity in Leaves of Primary Transformants of *Lolium multiflorum* Under VAC APO and ER Targeting Sequence

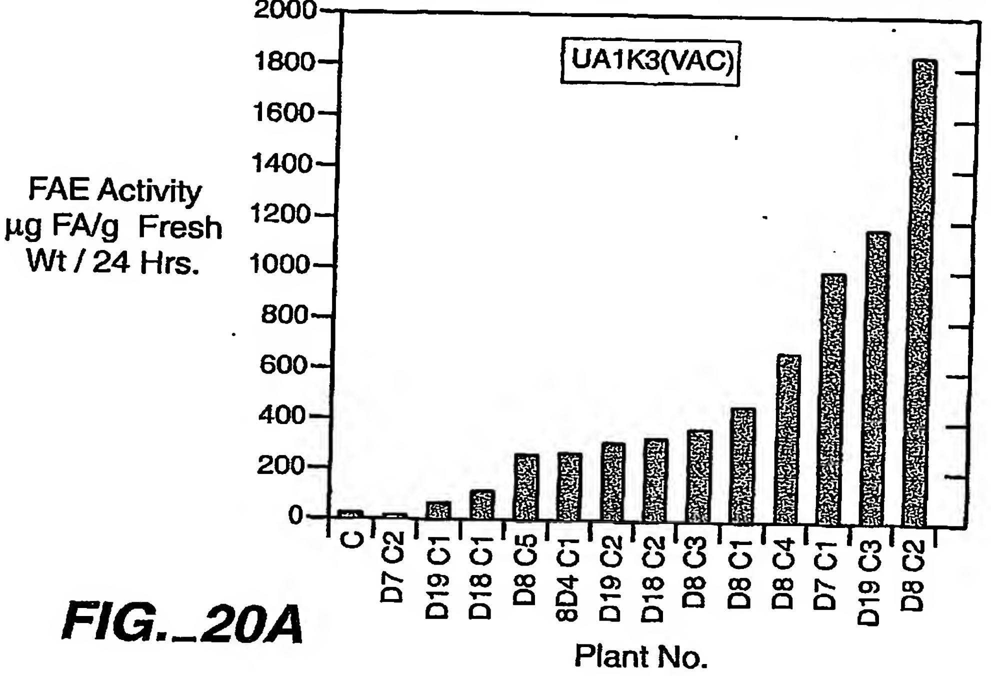


FIG._20A

FAE Activity in Leaves of Primary Transformants of *Lolium multiflorum* Under VAC APO and ER Targeting Sequence

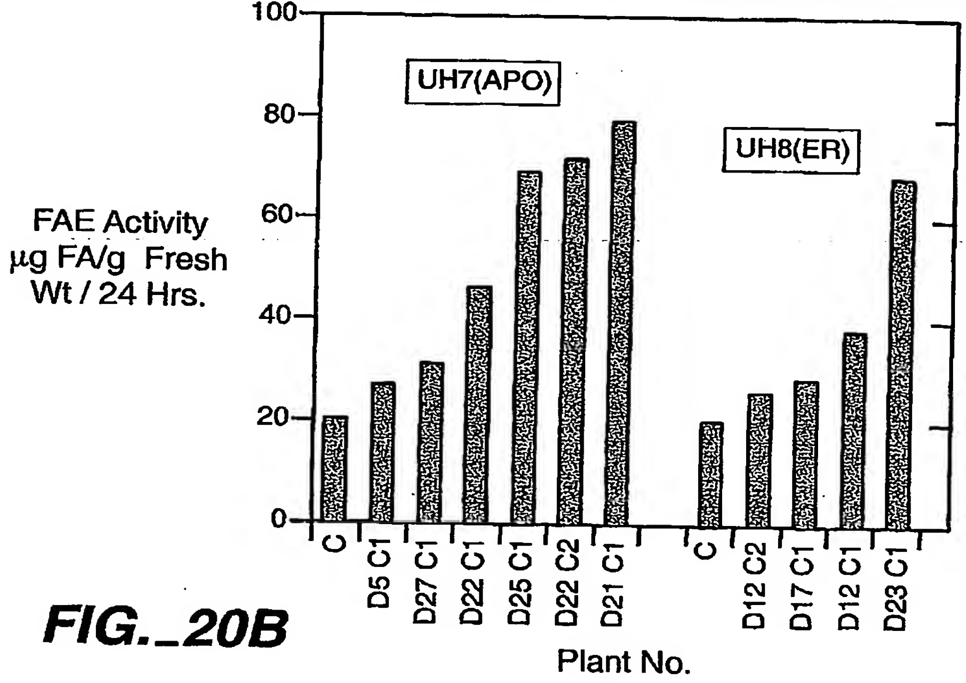


FIG._20B

Levels of Esterified Monomeric and Dimeric Hydroxycinnamicacids In *Festuca Arundinacea* Plants Expressing FAE Under VAC Targeting Sequence

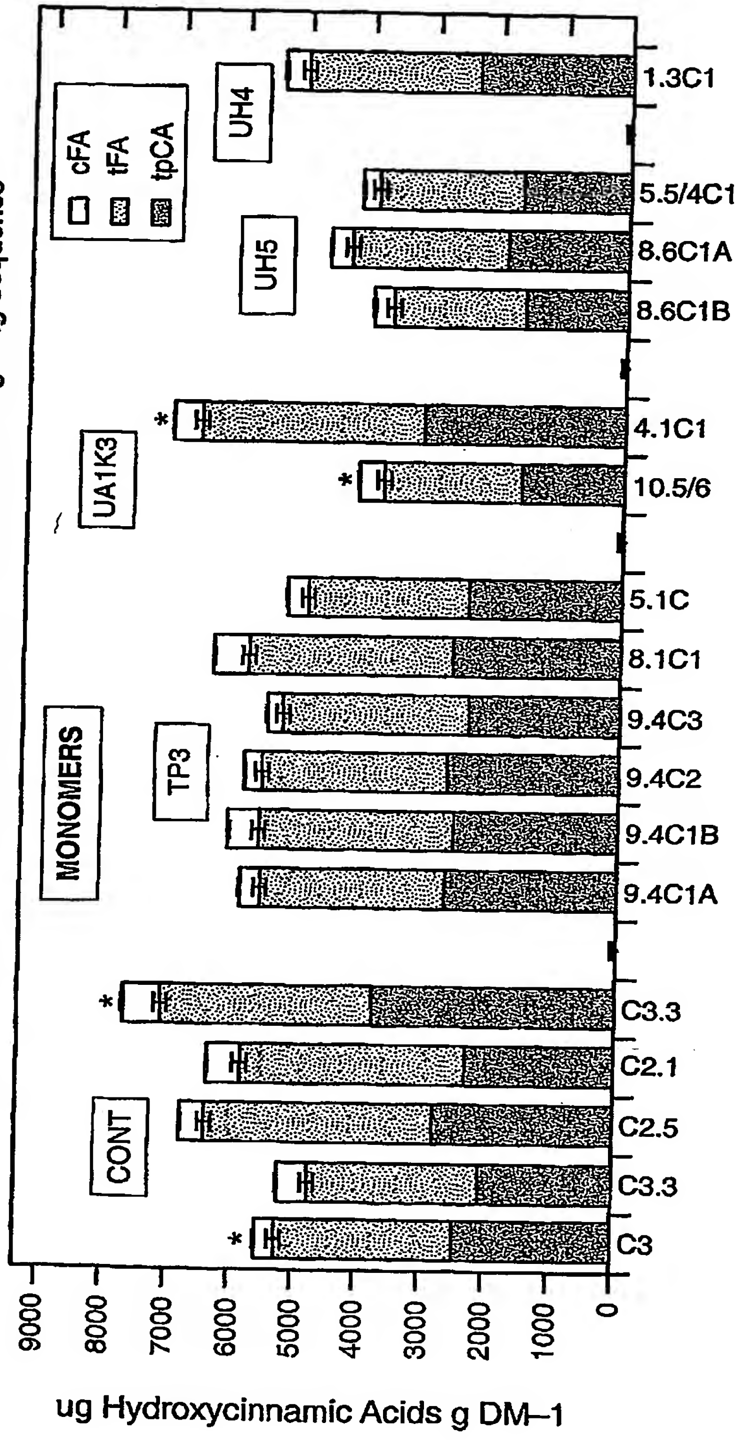
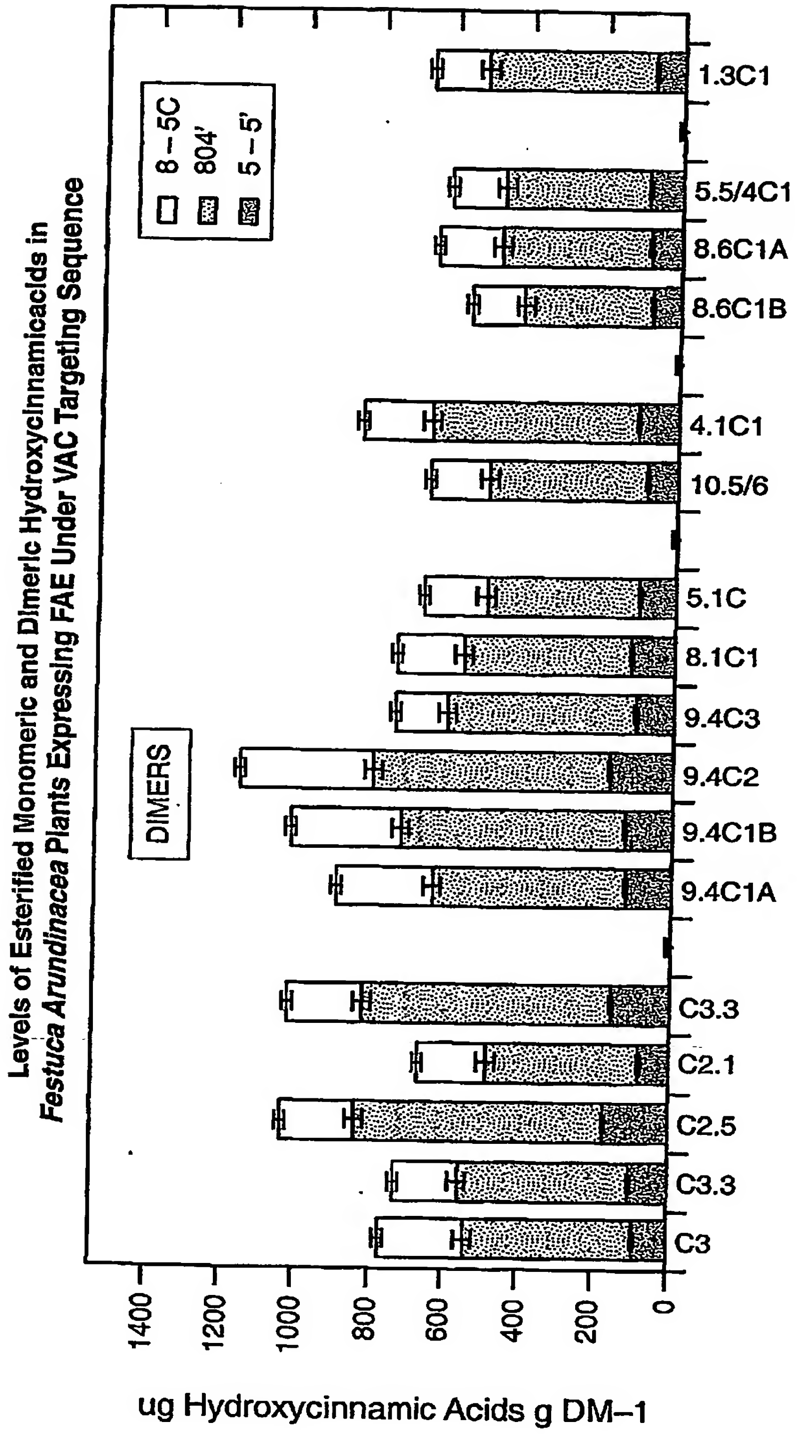


FIG._21A



Levels of Esterified Monomeric and Dimeric Hydroxycinnamicacids in *Festuca Arundinacea* Plants Expressing FAE Under VAC Targeting Sequence

FIG._21B

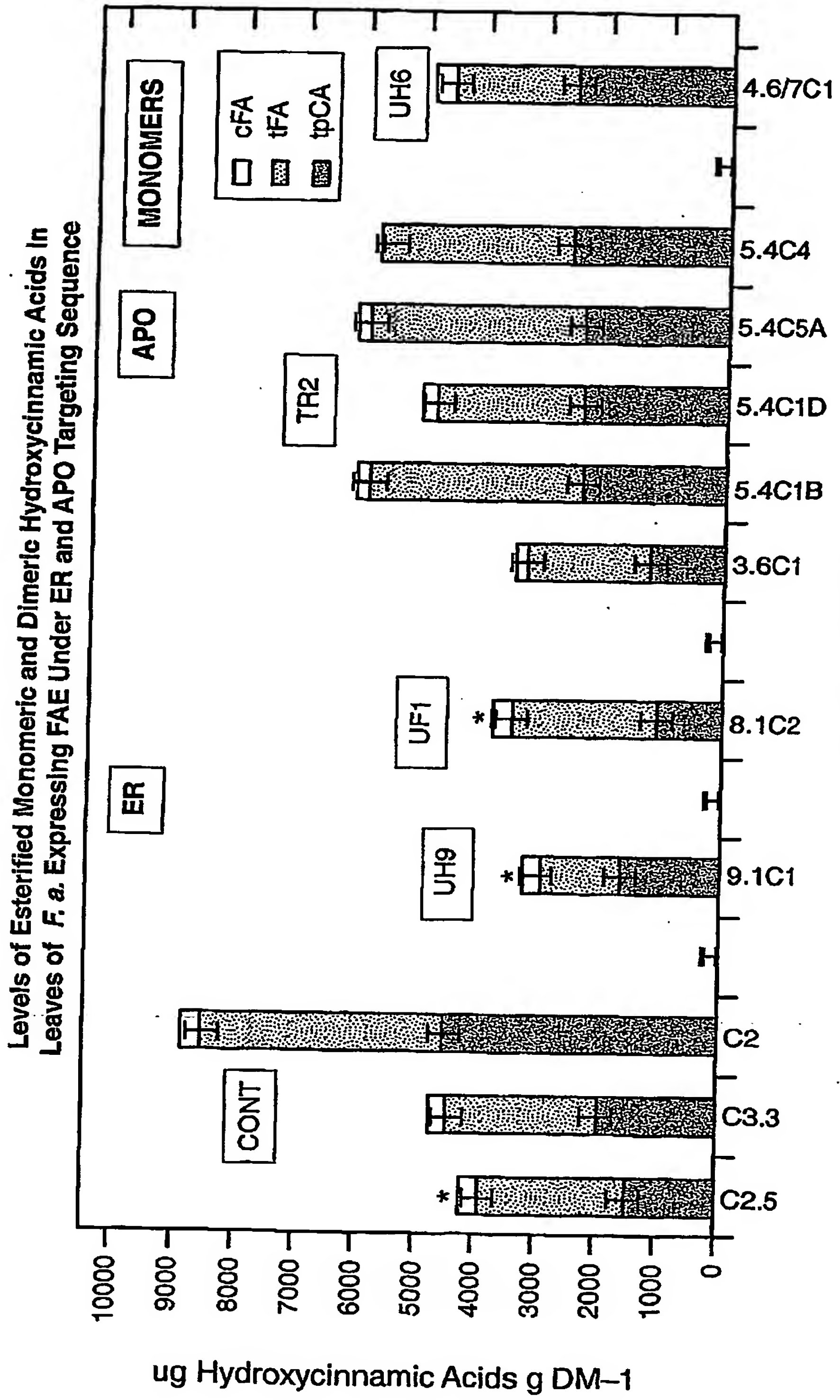

Levels of Esterified Monomeric and Dimeric Hydroxycinnamic Acids in Leaves of *F. a.* Expressing FAE Under ER and APO Targeting Sequence
MONOMERS
□ cFA
▨ tFA
▩ tpCA
CONT
ER
APO
UH9
UF1
TR2
UH6
*
C2.5
C3.3
C2
9.1C1
8.1C2
3.6C1
5.4C1B
5.4C1D
5.4C5A
5.4C4
4.6/7C1
0
1000
2000
3000
4000
5000
6000
7000
8000
9000
10000
ug Hydroxycinnamic Acids g DM–1

FIG._22A

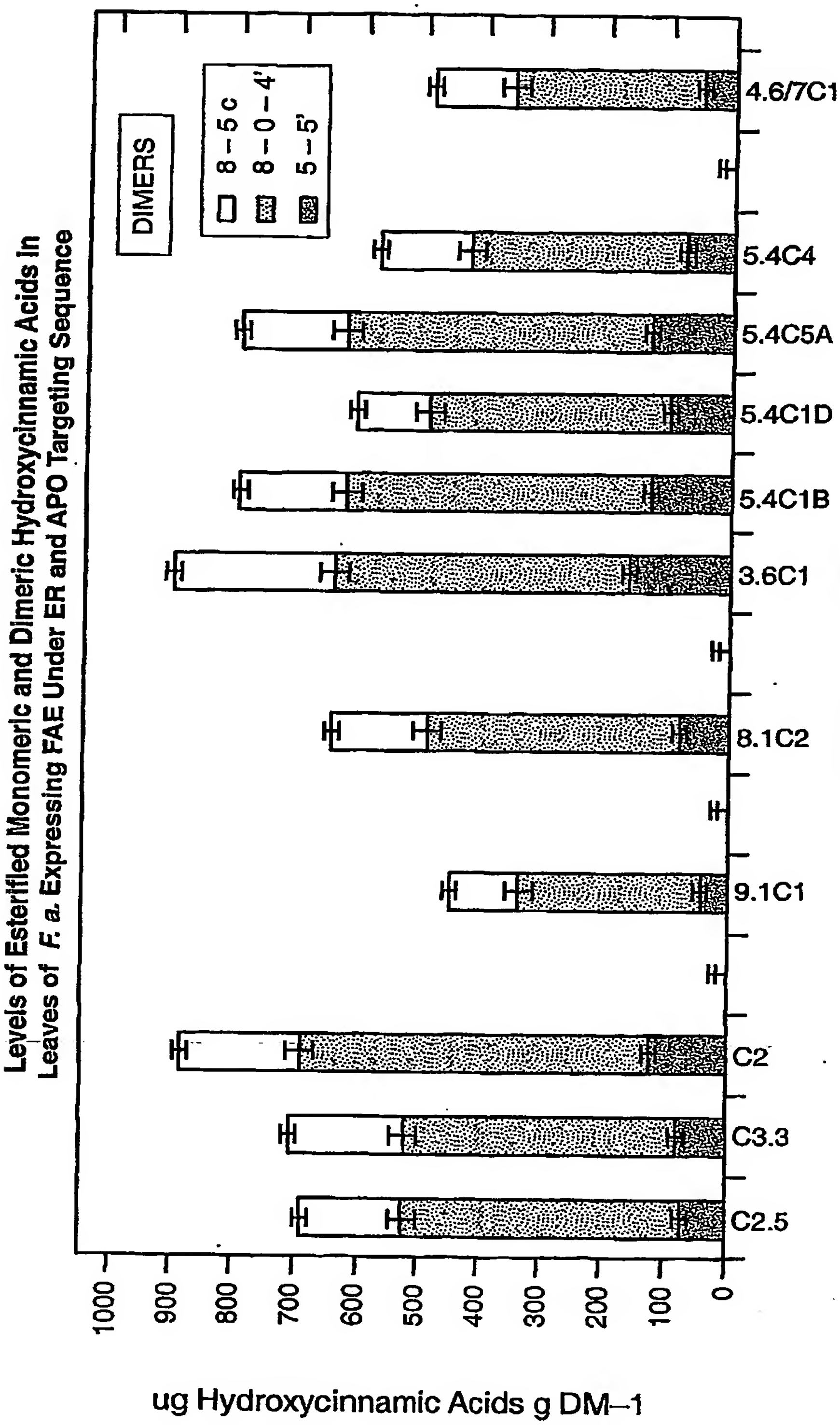

Levels of Esterified Monomeric and Dimeric Hydroxycinnamic Acids In Leaves of *F. a.* Expressing FAE Under ER and APO Targeting Sequence
DIMERS
8 – 5 c
8 – 0 – 4'
5 – 5'
4.6/7C1
5.4C4
5.4C5A
5.4C1D
5.4C1B
3.6C1
8.1C2
9.1C1
C2
C3.3
C2.5
1000
900
800
700
600
500
400
300
200
100
0
ug Hydroxycinnamic Acids g DM–1

FIG._22B

In Vitro Dry Mater Digestibility of Leaf Tissue of Mature *Festuca arundinacea* Plants Expressing FAE Under an Actin Promoter

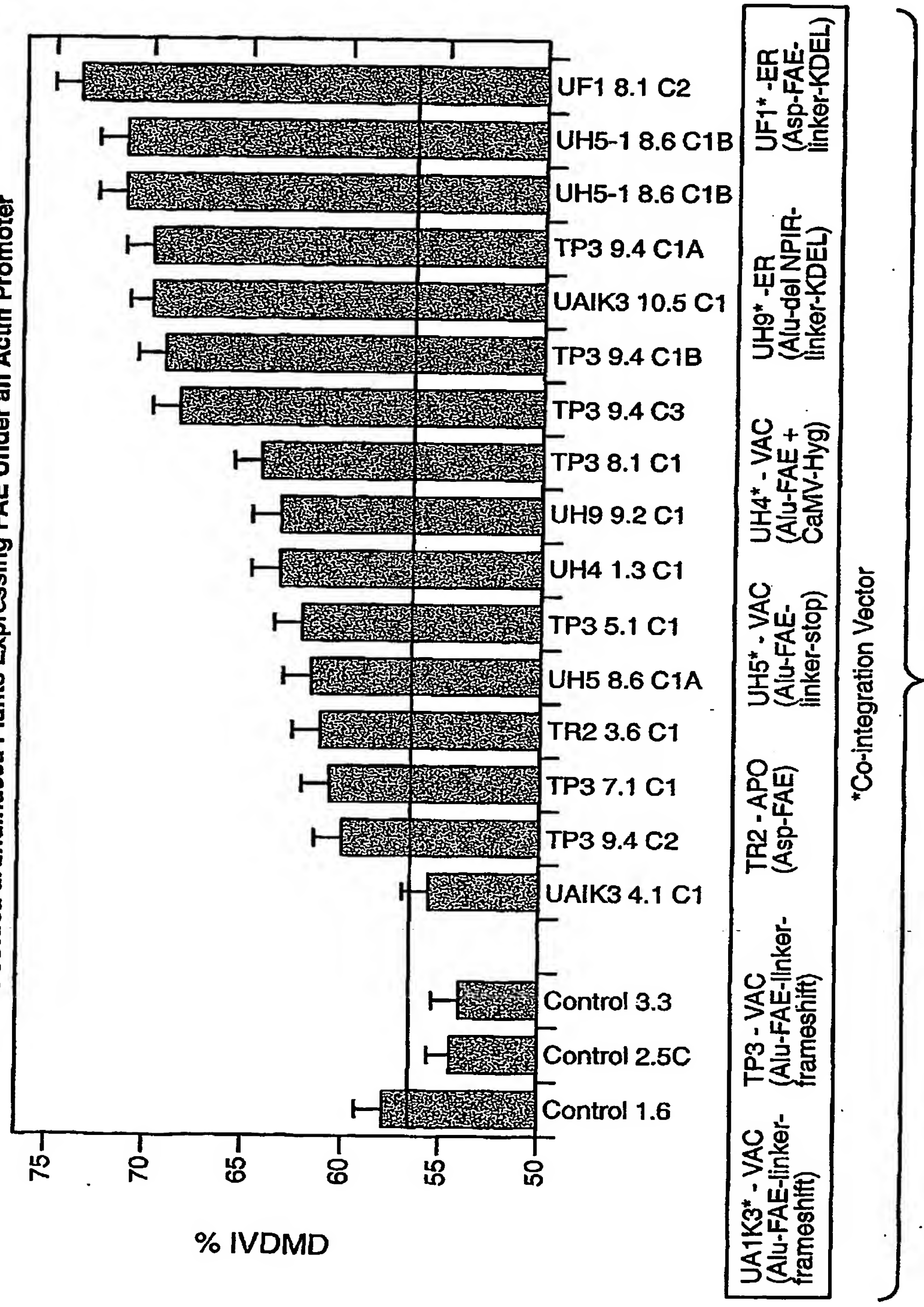


FIG._23

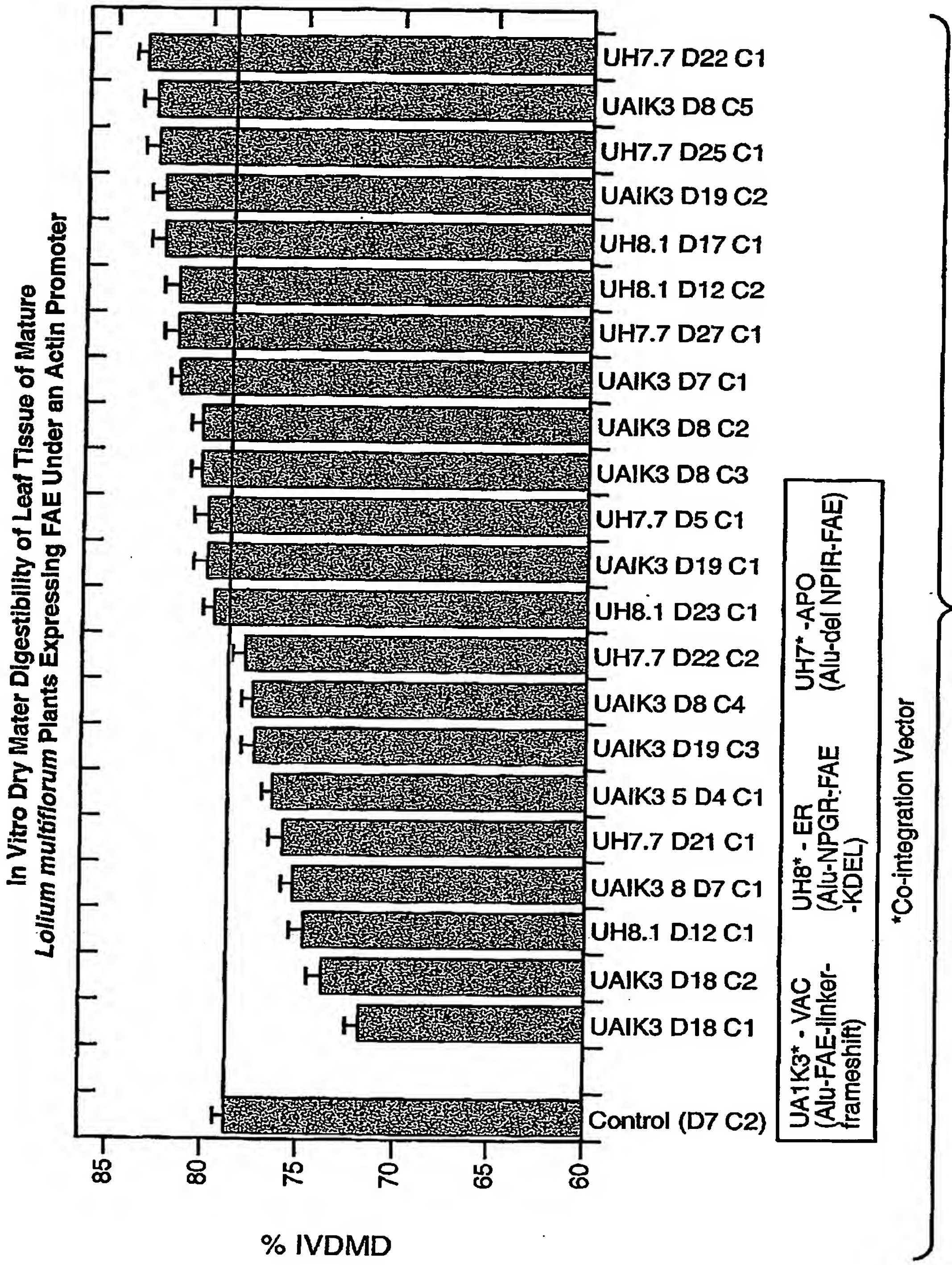

In Vitro Dry Mater Digestibility of Leaf Tissue of Mature
Lolium multiflorum Plants Expressing FAE Under an Actin Promoter
UH7.7 D22 C1
UAIK3 D8 C5
UH7.7 D25 C1
UAIK3 D19 C2
UH8.1 D17 C1
UH8.1 D12 C2
UH7.7 D27 C1
UAIK3 D7 C1
UAIK3 D8 C2
UAIK3 D8 C3
UH7.7 D5 C1
UAIK3 D19 C1
UH8.1 D23 C1
UH7.7 D22 C2
UAIK3 D8 C4
UAIK3 D19 C3
UAIK3 5 D4 C1
UH7.7 D21 C1
UAIK3 8 D7 C1
UH8.1 D12 C1
UAIK3 D18 C2
UAIK3 D18 C1
Control (D7 C2)
85
80
75
70
65
60
% IVDMD
UA1K3* - VAC (Alu-FAE-linker-frameshift)
UH8* - ER (Alu-NPGR-FAE -KDEL)
UH7* -APO (Alu-del NPIR-FAE)
*Co-integration Vector

FIG._24

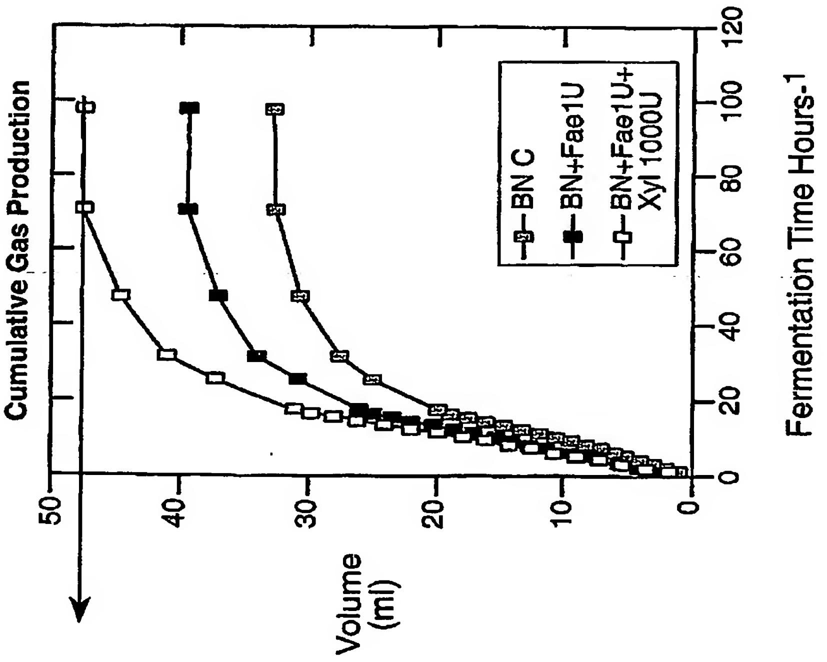


FIG._25A

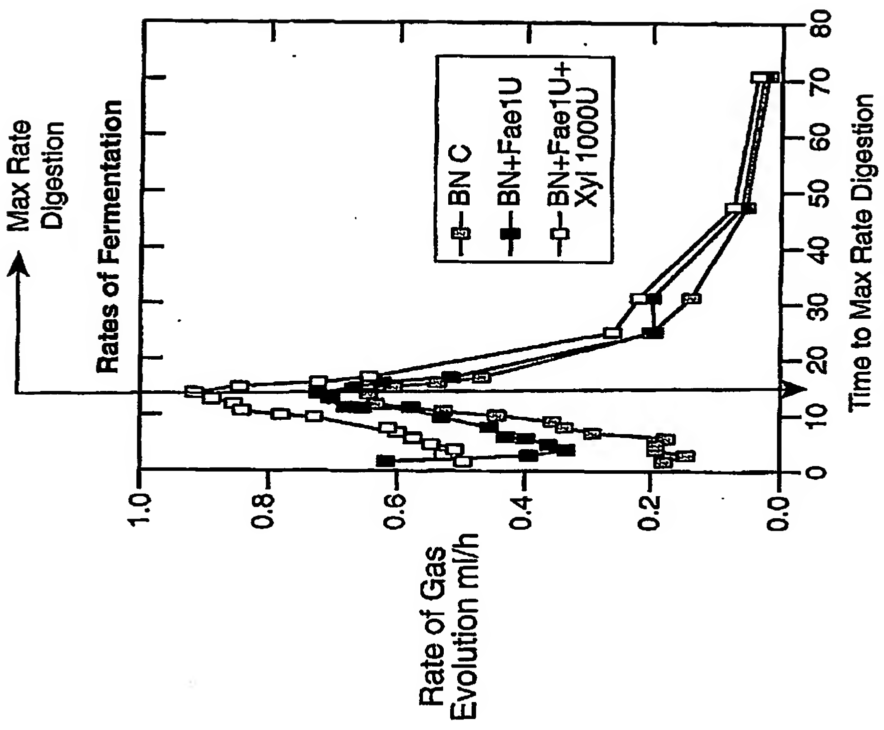


FIG._25B

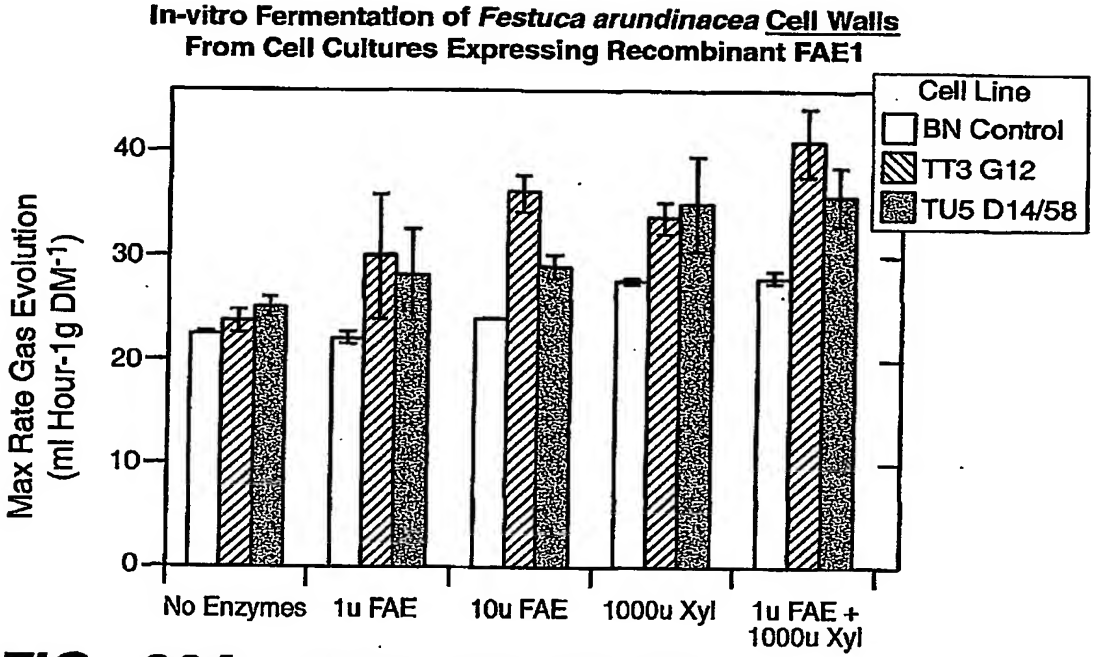


FIG._26A Maximum Rate of Digestion

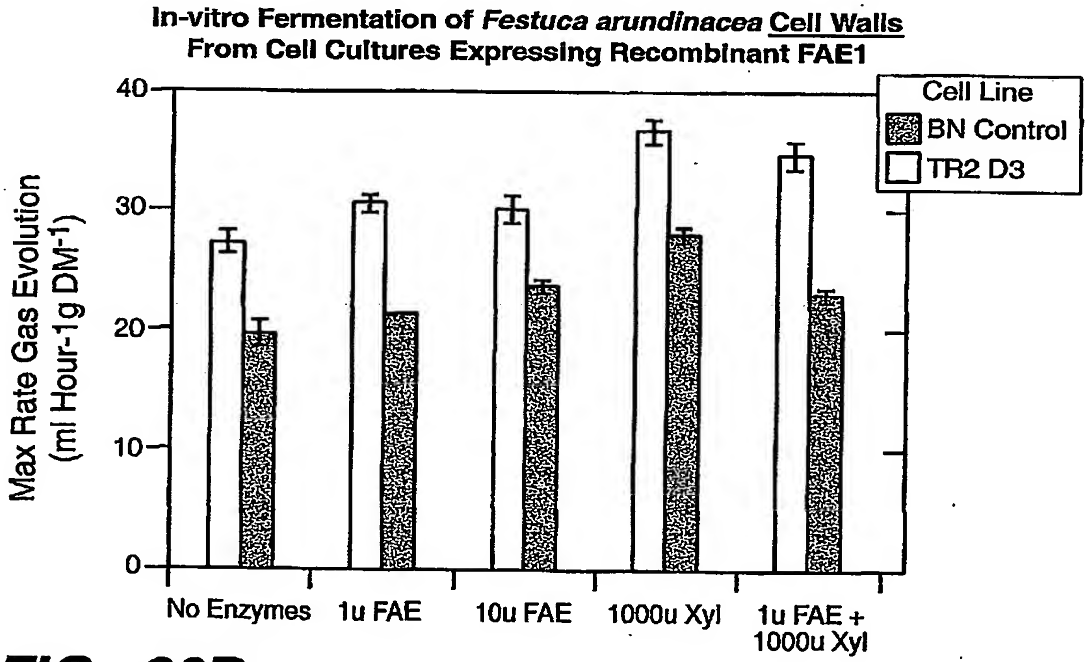


FIG._26B Maximum Rate of Digestion

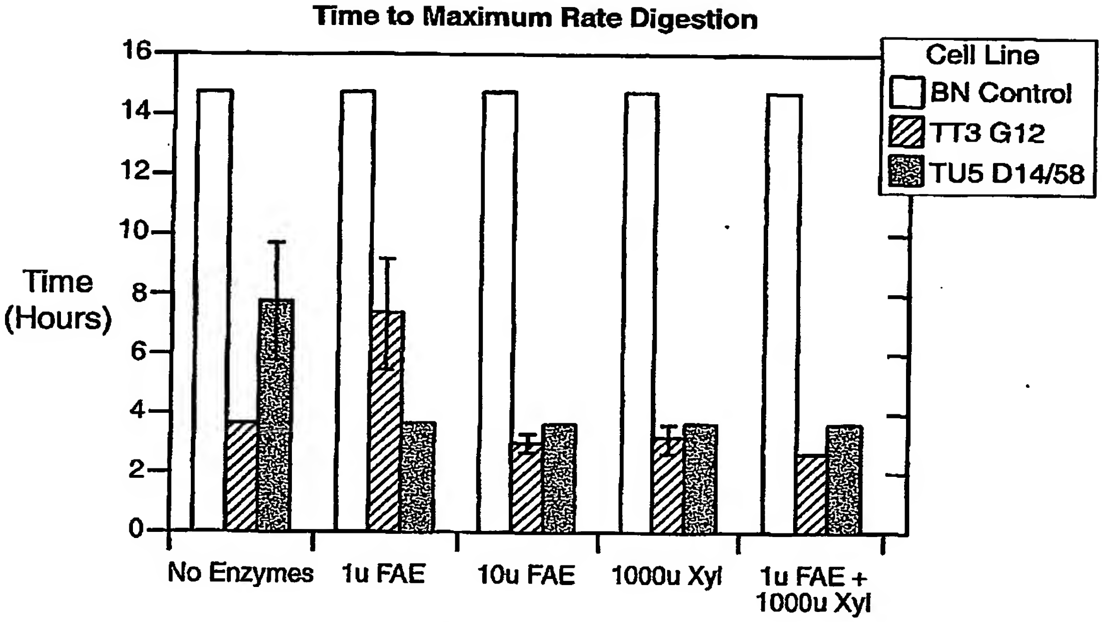


FIG._27A

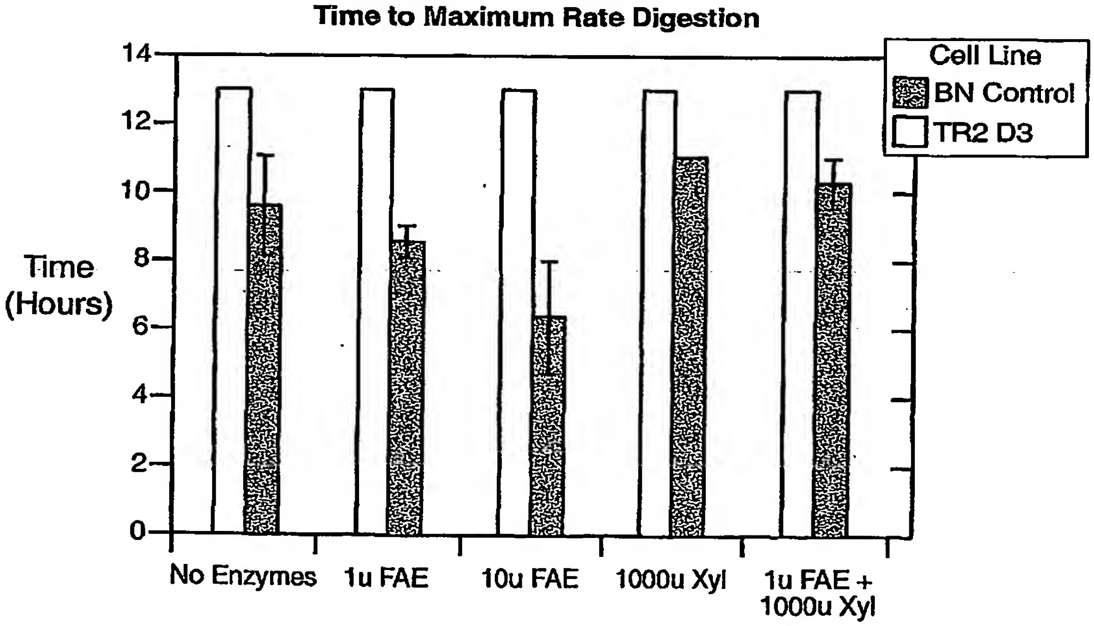


FIG._27B

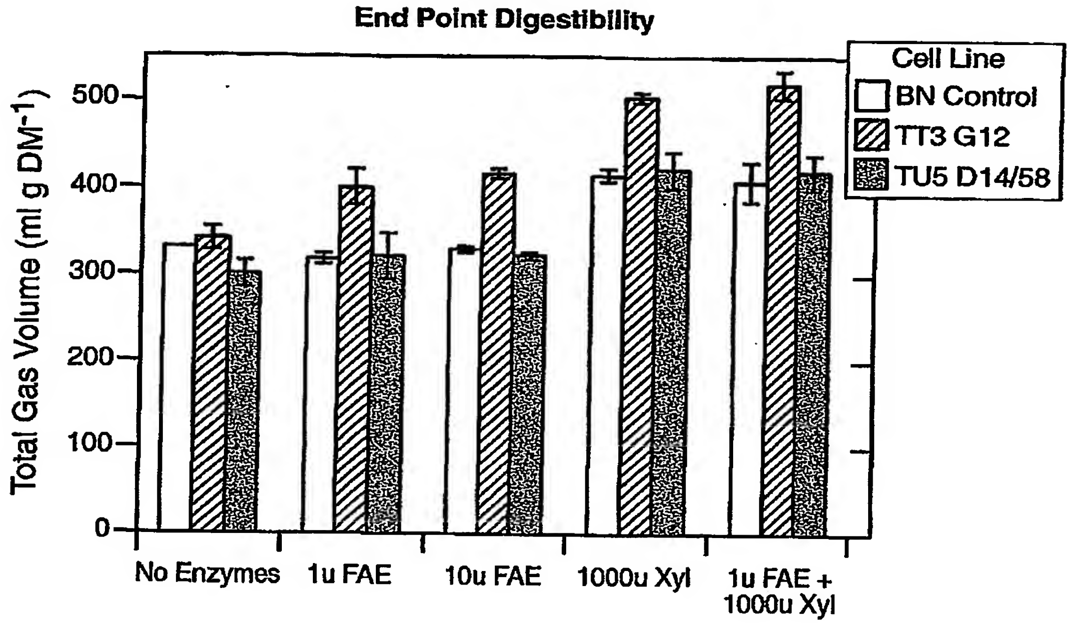


FIG._28A

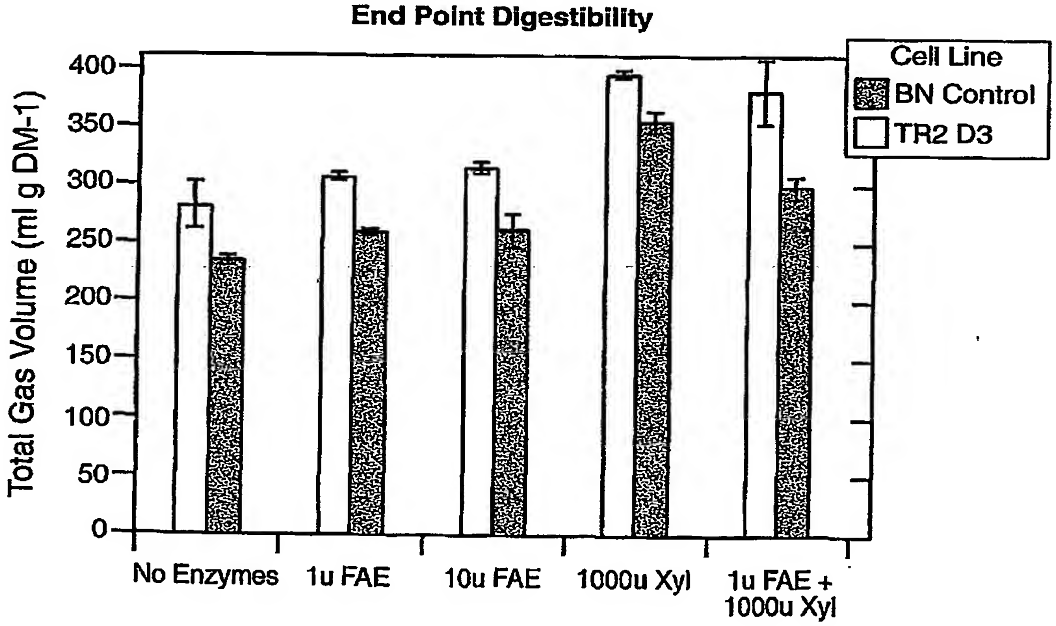


FIG._28B

Kinetics of FAE Activity by Ferulic Acid Release from Cell Wall under Self Digestion in *Festuca arundinacea* and Stimulation by Xylanase

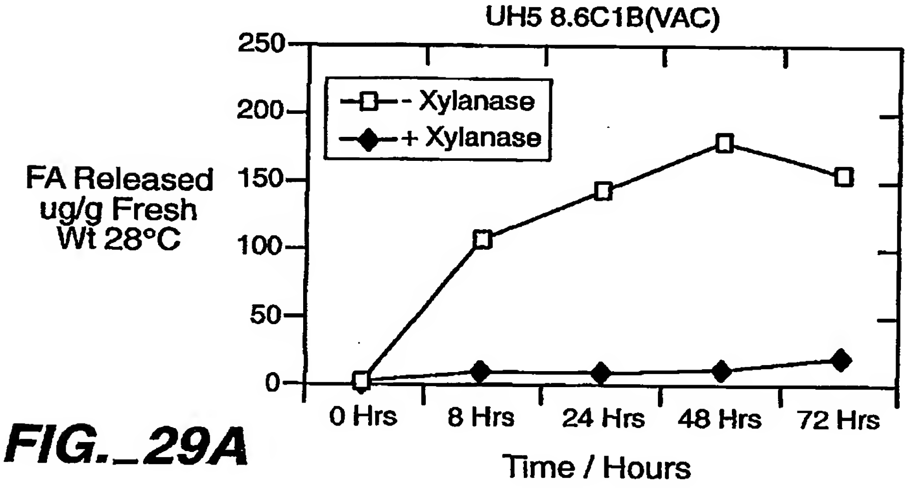


FIG._29A

Kinetics of FAE Activity by Ferulic Acid Release from Cell Wall under Self Digestion in *Festuca arundinacea* and Stimulation by Xylanase.

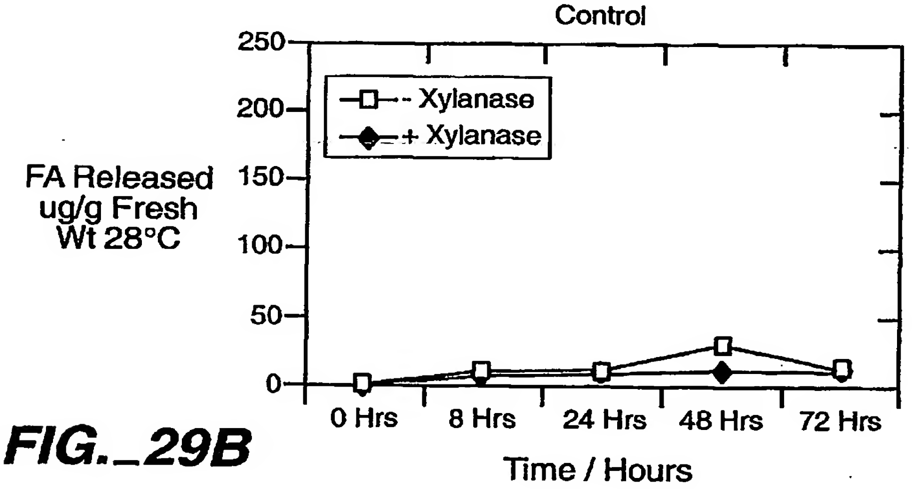


FIG._29B

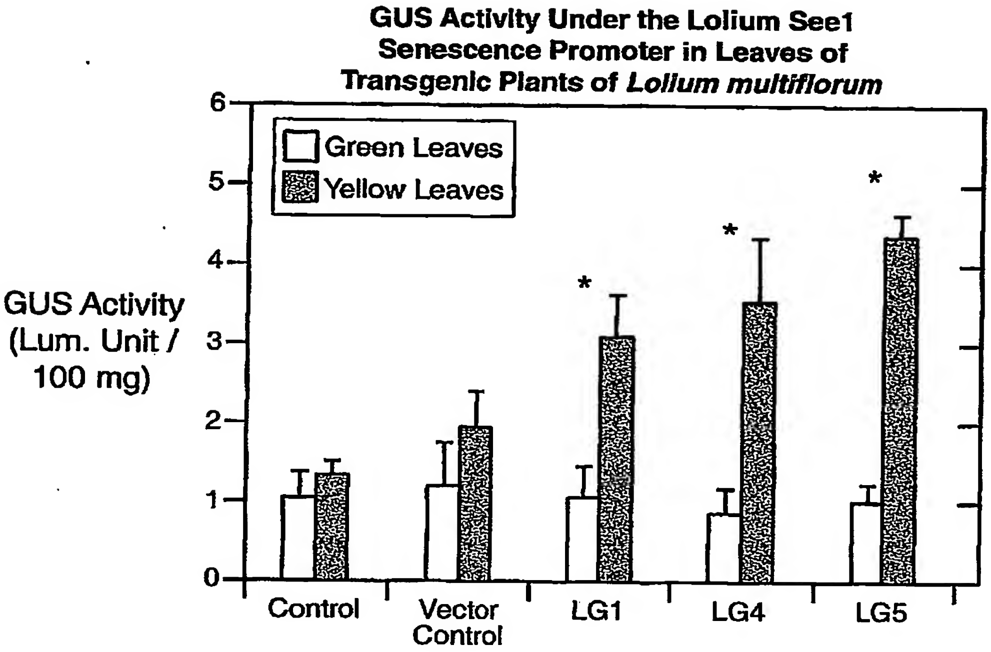

GUS Activity Under the Lolium See1 Senescence Promoter in Leaves of Transgenic Plants of *Lolium multiflorum*
□ Green Leaves
▨ Yellow Leaves
GUS Activity (Lum. Unit / 100 mg)
6
5
4
3
2
1
0
*
*
*
Control
Vector Control
LG1
LG4
LG5

FIG._30

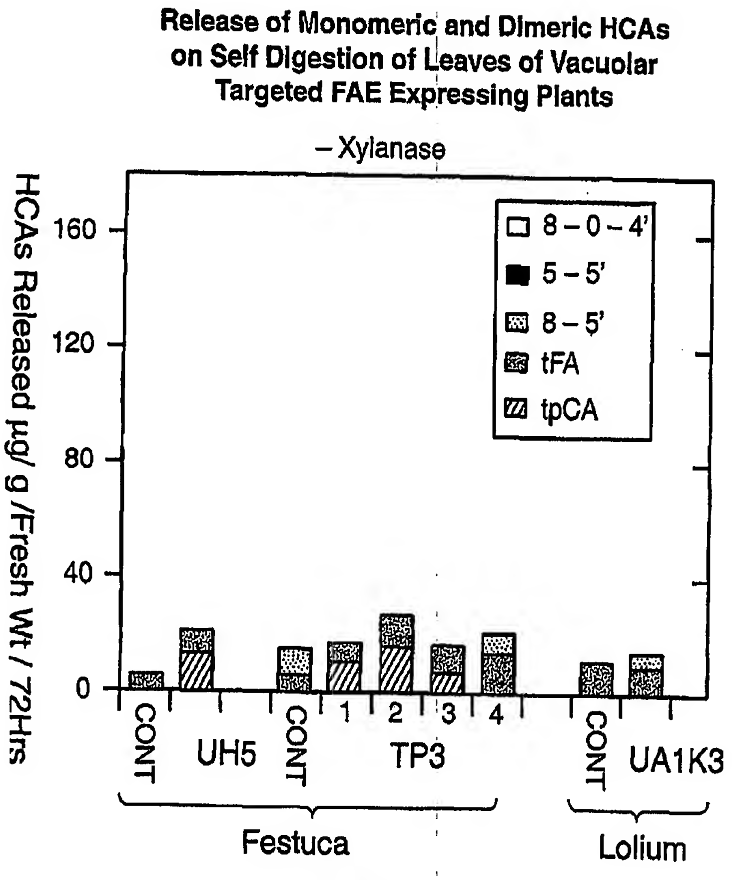


FIG._31A

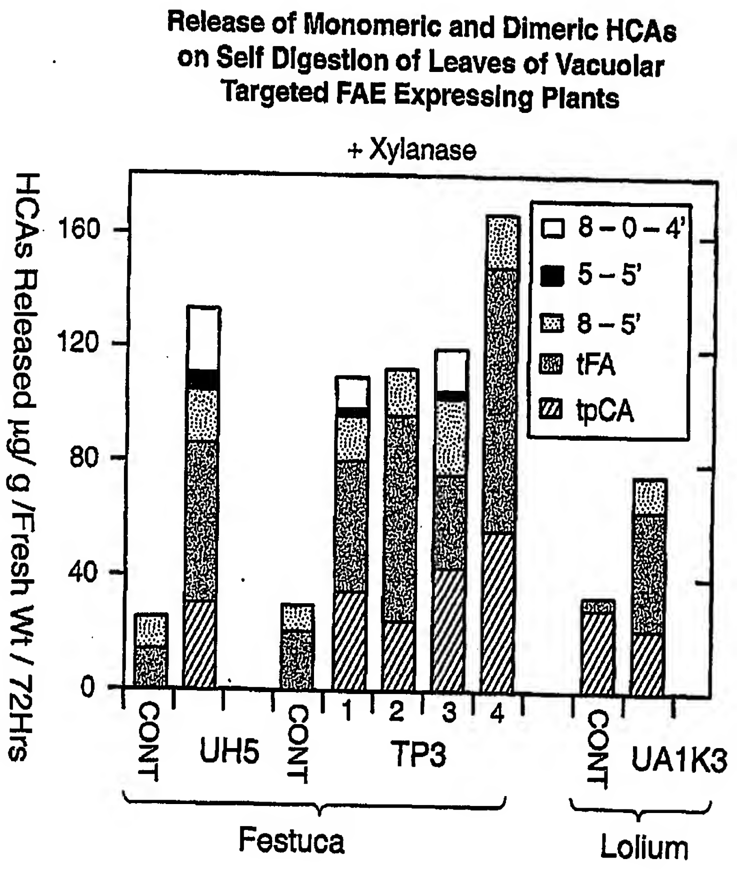


FIG._31B

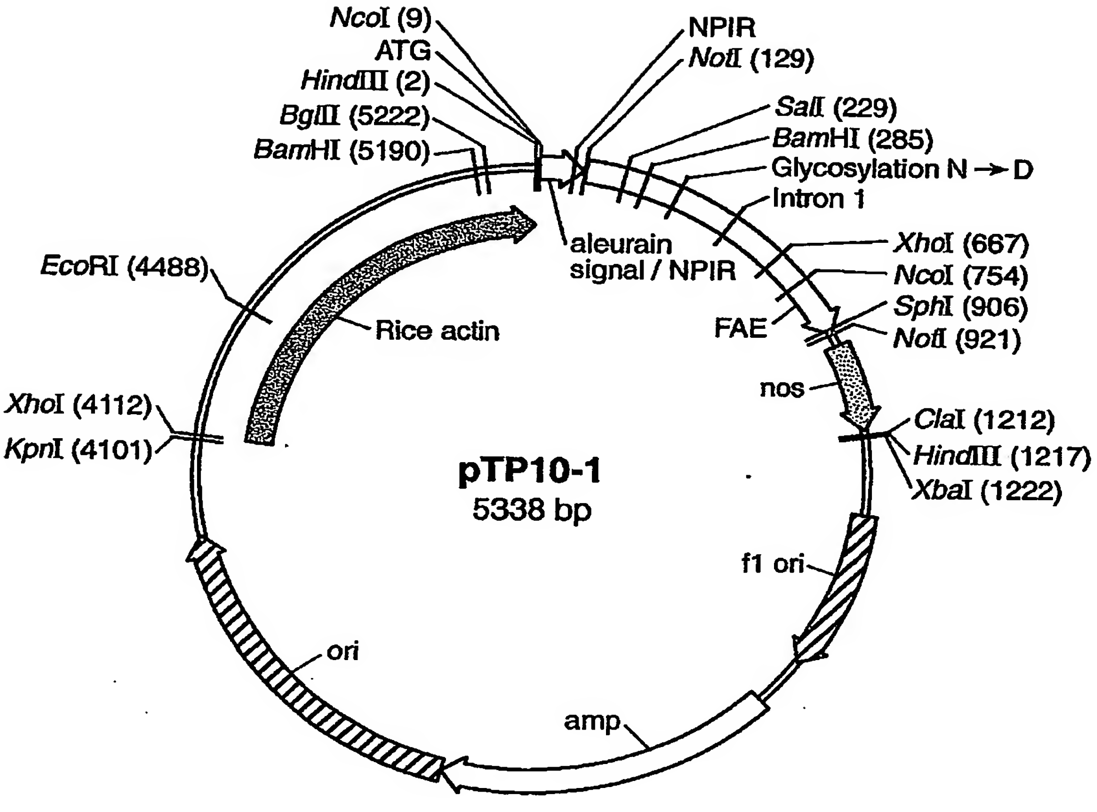

NcoI (9)
ATG
HindIII (2)
BglII (5222)
BamHI (5190)
NPIR
NotI (129)
SalI (229)
BamHI (285)
Glycosylation N → D
Intron 1
aleurain signal / NPIR
XhoI (667)
NcoI (754)
SphI (906)
NotI (921)
FAE
EcoRI (4488)
Rice actin
nos
XhoI (4112)
KpnI (4101)
ClaI (1212)
HindIII (1217)
XbaI (1222)
pTP10-1
5338 bp
f1 ori
ori
amp

FIG._32A

```
             NcoI
           ~~~~~~~~
    HindIII
    ~~~~~~
               M  A  H  A   R  V  L   L  L  A   L  A  V  L   A  T  A   A  V  A   V
  1 AAGCTTACCA TGGCCCACGC CCGCGTCCTC CTCCTGGCGC TCGCCGTGCT GGCCACGGCC GCCGTCGCCG

                                        NPIR                 NotI
                                    ~~~~~~~~~~~~~~        ~~~~~~~~~
    ·  A  S  S   S  S  F   A  D  S   N  P  I  R   P  V  T   D  R  A   A  A  S  T ·
 71 TCGCCTCCTC CTCCTCCTTC GCCGACTCCA ACCCGATCCG GCCCGTCACC GACCGCGCGG CCGCCTCCAC
    · Q  G  I   S  E  D   L  Y  S  R   L  V  E   M  A  T   I  S  Q  A   A  Y  A
141 GCAGGGCATC TCCGAAGACC TCTACAGCCG TTTAGTCGAA ATGGCCACTA TCTCCCAAGC TGCCTACGCC

                      SalI
                    ~~~~~~~~
     D  L  C   N  I  P  S   T  I  I   K  G  E   K  I  Y  N   S  Q  T   D  I  N   G
211 GACCTGTGCA ACATTCCGTC GACTATTATC AAGGGAGAGA AAATTTACAA TTCTCAAACT GACATTAACG

      BamHI
      ~~~~~~~
    ·  W  I  L   R  D  D   S  S  K   E  I  I  T   V  F  R   G  T  G   S  D  T  N ·
281 GATGGATCCT CCGCGACGAC AGCAGCAAAG AAATAATCAC CGTCTTCCGT GGCACTGGTA GTGATACGAA

                     Glycosylation
                     ~~~~
    · L  Q  L   D  T  D   Y  T  L  T   P  F  D   T  L  P   Q  C  N  G   C  E  V
351 TCTACAACTC GATACTGACT ACACCCTCAC GCCTTTCGAC ACCCTACCAC AATGCAACGG TTGTGAAGTA
    H  G  G  Y   Y  I  G   W  V  S   V  Q  D  Q   V  E  S   L  V  K   Q  Q  V  S
421 CACGGTGGAT ATTATATTGG ATGGGTCTCC GTCCAGGACC AAGTCGAGTC GCTTGTCAAA CAGCAGGTTA
    · Q  Y  P   D  Y  A   L  T  V  T   G  H  X   L  G  A   S  L  A  A   L  T  A ·
491 GCCAGTATCC GGACTACGCG CTGACCGTGA CCGGCCACKC CCTCGGCGCC TCCCTGGCGG CACTCACTGC
    ·A  Q  L   S  A  T  Y   D  N  I   R  L  Y   T  F  G  E   P  R  S   G  N  Q
561 CGCCCAGCTG TCTGCGACAT ACGACAACAT CCGCCTGTAC ACCTTCGGCG AACCGCGCAG CGGCAATCAG
```

FIG._32B

```
                                             XhoI
                                           ~~~~~~~~
       A  F  A   S  Y  M  N   D  A  F   Q  A  S   S  P  D  T   T  Q  Y   F  R  V   T
 631  GCCTTCGCGT CGTACATGAA CGATGCCTTC CAAGCCTCGA GCCCAGATAC GACGCAGTAT TTCCGGGTCA

                                                                NcoI
                                                              ~~~~~~~~
      ·  H  A  N   D  G  I   P  N  L   P  P  V  E   Q  G  Y   A  H  G   G  V  E  Y ·
 701  CTCATGCCAA CGACGGCATC CCAAACCTGC CCCCGGTGGA GCAGGGGTAC GCCCATGGCG GTGTAGAGTA
       ·W  S  V   D  P  Y   S  A  Q  N   T  F  V   C  T  G   D  E  V   Q  C  C   E
 771  CTGGAGCGTT GATCCTTACA GCGCCCAGAA CACATTTGTC TGCACTGGGG ATGAAGTGCA GTGCTGTGAG

                                                                         SphI
                                                                         ~~~~
       A  Q  G   G  Q  G  V   N  N  A   H  T  T   Y  F  G  M   T  S  G   A  C  T   W
 841  GCCCAGGGCG GACAGGGTGT GAATAATGCG CACACGACTT ATTTTGGGAT GACGAGCGGC GCATGCACCT

                NotI                   KDEL
             ~~~~~~~~~~          ~~~~~~~~~~~~~~~
      ·  P  V  A   A  A  E   T  T  E   G  *
 911  GGCCGGTCGC GGCCGCGGAA ACCACTGAAG GATGAGCTGT AAAGAAGCAG ATCGTTCAAA CATTTGGCAA
 981  TAAAGTTTCT TAAGATTGAA TCCTGTTGCC GGTCTTGCGA TGATTATCAT ATAATTTCTG TTGAATTACG
1051  TTAAGCATGT AATAATTAAC ATGTAATGCA TGACGTTATT TATGAGATGG GTTTTTATGA TTAGAGTCCC
1121  GCAATTATAC ATTTAATACG CGATAGAAAA CAAAATATAG CGCGCAAACT AGGATAAATT ATCGCGCGCG

                                 HindIII
                                 ~~~~~~~~
                           ClaI         XbaI
                          ~~~~~~~      ~~~~~~~
1191  GTGTCATCTA TGTTACTAGA TCGATAAGCT TCTAGAGCGG CCGGTGGAGC TCCAATTCGC CCTATAGTGA
1261  GTCGTATTAC GCGCGCTCAC TGGCCGTCGT TTTACAACGT CGTGACTGGG AAAACCCTGG CGTTACCCAA
1331  CTTAATCGCC TTGCAGCACA TCCCCCTTTC GCCAGCTGGC GTAATAGCGA AGAGGCCCGC ACCGATCGCC
1401  CTTCCCAACA GTTGCGCAGC CTGAATGGCG AATGGGACGC GCCCTGTAGC GGCGCATTAA GCGCGGCGGG
```

FIG._32C

```
1471  TGTGGTGGTT ACGCGCAGCG TGACCGCTAC ACTTGCCAGC GCCCTAGCGC CCGCTCCTTT CGCTTTCTTC
1541  CCTTCCTTTC TCGCCACGTT CGCCGGCTTT CCCCGTCAAG CTCTAAATCG GGGGCTCCCT TTAGGGTTCC
1611  GATTTAGTGC TTTACGGCAC CTCGACCCCA AAAAACTTGA TTAGGGTGAT GGTTCACGTA GTGGGCCATC
1681  GCCCTGATAG ACGGTTTTTC GCCCTTTGAC GTTGGAGTCC ACGTTCTTTA ATAGTGGACT CTTGTTCCAA
1751  ACTGGAACAA CACTCAACCC TATCTCGGTC TATTCTTTTG ATTTATAAGG GATTTTGCCG ATTTCGGCCT
1821  ATTGGTTAAA AAATGAGCTG ATTTAACAAA AATTTAACGC GAATTTTAAC AAAATATTAA CGCTTACAAT
1891  TTAGGTGGCA CTTTTCGGGG AAATGTGCGC GGAACCCCTA TTTGTTTATT TTTCTAAATA CATTCAAATA
1961  TGTATCCGCT CATGAGACAA TAACCCTGAT AAATGCTTCA ATAATATTGA AAAAGGAAGA GTATGAGTAT
2031  TCAACATTTC CGTGTCGCCC TTATTCCCTT TTTTGCGGCA TTTTGCCTTC CTGTTTTTGC TCACCCAGAA
2101  ACGCTGGTGA AAGTAAAAGA TGCTGAAGAT CAGTTGGGTG CACGAGTGGG TTACATCGAA CTGGATCTCA
2171  ACAGCGGTAA GATCCTTGAG AGTTTTCGCC CCGAAGAACG TTTTCCAATG ATGAGCACTT TTAAAGTTCT
2241  GCTATGTGGC GCGGTATTAT CCCGTATTGA CGCCGGGCAA GAGCAACTCG GTCGCCGCAT ACACTATTCT
2311  CAGAATGACT TGGTTGAGTA CTCACCAGTC ACAGAAAAGC ATCTTACGGA TGGCATGACA GTAAGAGAAT
2381  TATGCAGTGC TGCCATAACC ATGAGTGATA ACACTGCGGC CAACTTACTT CTGACAACGA TCGGAGGACC
2451  GAAGGAGCTA ACCGCTTTTT TGCACAACAT GGGGGATCAT GTAACTCGCC TTGATCGTTG GGAACCGGAG
2521  CTGAATGAAG CCATACCAAA CGACGAGCGT GACACCACGA TGCCTGTAGC AATGGCAACA ACGTTGCGCA
2591  AACTATTAAC TGGCGAACTA CTTACTCTAG CTTCCCGGCA ACAATTAATA GACTGGATGG AGGCGGATAA
2661  AGTTGCAGGA CCACTTCTGC GCTCGGCCCT TCCGGCTGGC TGGTTTATTG CTGATAAATC TGGAGCCGGT
2731  GAGCGTGGGT CTCGCGGTAT CATTGCAGCA CTGGGGCCAG ATGGTAAGCC CTCCCGTATC GTAGTTATCT
2801  ACACGACGGG GAGTCAGGCA ACTATGGATG AACGAAATAG ACAGATCGCT GAGATAGGTG CCTCACTGAT
2871  TAAGCATTGG TAACTGTCAG ACCAAGTTTA CTCATATATA CTTTAGATTG ATTTAAAACT TCATTTTTAA
2941  TTTAAAAGGA TCTAGGTGAA GATCCTTTTT GATAATCTCA TGACCAAAAT CCCTTAACGT GAGTTTTCGT
3011  TCCACTGAGC GTCAGACCCC GTAGAAAAGA TCAAAGGATC TTCTTGAGAT CCTTTTTTTC TGCGCGTAAT
3081  CTGCTGCTTG CAAACAAAAA AACCACCGCT ACCAGCGGTG GTTTGTTTGC CGGATCAAGA GCTACCAACT
3151  CTTTTTCCGA AGGTAACTGG CTTCAGCAGA GCGCAGATAC CAAATACTGT CCTTCTAGTG TAGCCGTAGT
3221  TAGGCCACCA CTTCAAGAAC TCTGTAGCAC CGCCTACATA CCTCGCTCTG CTAATCCTGT TACCAGTGGC
3291  TGCTGCCAGT GGCGATAAGT CGTGTCTTAC CGGGTTGGAC TCAAGACGAT AGTTACCGGA TAAGGCGCAG
3361  CGGTCGGGCT GAACGGGGGG TTCGTGCACA CAGCCCAGCT TGGAGCGAAC GACCTACACC GAACTGAGAT
3431  ACCTACAGCG TGAGCTATGA GAAAGCGCCA CGCTTCCCGA AGGGAGAAAG GCGGACAGGT ATCCGGTAAG
3501  CGGCAGGGTC GGAACAGGAG AGCGCACGAG GGAGCTTCCA GGGGGAAACG CCTGGTATCT TTATAGTCCT
3571  GTCGGGTTTC GCCACCTCTG ACTTGAGCGT CGATTTTTGT GATGCTCGTC AGGGGGGCGG AGCCTATGGA
3641  AAAACGCCAG CAACGCGGCC TTTTTACGGT TCCTGGCCTT TTGCTGGCCT TTTGCTCACA TGTTCTTTCC
3711  TGCGTTATCC CCTGATTCTG TGGATAACCG TATTACCGCC TTTGAGTGAG CTGATACCGC TCGCCGCAGC
3781  CGAACGACCG AGCGCAGCGA GTCAGTGAGC GAGGAAGCGG AAGAGCGCCC AATACGCAAA CCGCCTCTCC
3851  CCGCGCGTTG GCCGATTCAT TAATGCAGCT GGCACGACAG GTTTCCCGAC TGGAAAGCGG GCAGTGAGCG
```

FIG._32D

```
3921  CAACGCAATT AATGTGAGTT AGCTCACTCA TTAGGCACCC CAGGCTTTAC ACTTTATGCT TCCGGCTCGT
3991  ATGTTGTGTG GAATTGTGAG CGGATAACAA TTTCACACAG GAAACAGCTA TGACCATGAT TACGCCAAGC
                                             KpnI             XhoI
                                            ~~~~~~~~          ~~~~~~~~
4061  GCGCAATTAA CCCTCACTAA AGGGAACAAA AGCTGGGTAC CGGGCCCCCC CTCGAGGTCA TTCATATGCT
4131  TGAGAAGAGA GTCGGGATAG TCCAAAATAA AACAAAGGTA AGATTACCTG GTCAAAAGTG AAAACATCAG
4201  TTAAAAGGTG GTATAAGTAA AATATCGGTA ATAAAAGGTG GCCCAAAGTG AAATTTACTC TTTTCTACTA
4271  TTATAAAAAT TGAGGATGTT TTGTCGGTAC TTTGATACGT CATTTTTGTA TGAATTGGTT TTTAAGTTTA
4341  TTCGCGATTT GGAAATGCAT ATCTGTATTT GAGTCGGTTT TTAAGTTCGT TGCTTTTGTA AATACAGAGG
4411  GATTTGTATA AGAAATATCT TTAAAAAACC CATATGCTAA TTTGACATAA TTTTTGAGAA AAATATATAT
            EcoRI
            ~~~~~~~~
4481  TCAGGCGAAT TCCACAATGA ACAATAATAA GATTAAAATA GCTTGCCCCC GTTGCAGCGA TGGGTATTTT
4551  TTCTAGTAAA ATAAAAGATA AACTTAGACT CAAAACATTT ACAAAAACAA CCCCTAAAGT CCTAAAGCCC
4621  AAAGTGCTAT GCACGATCCA TAGCAAGCCC AGCCCAACCC AACCCAACCC AACCCACCCC AGTGCAGCCA
4691  ACTGGCAAAT AGTCTCCACC CCCGGCACTA TCACCGTGAG TTGTCCGCAC CACCGCACGT CTCGCAGCCA
4761  AAAAAAAAAA AAGAAAGAAA AAAAAGAAAA AGAAAAACAG CAGGTGGGTC CGGGTCGTGG GGGCCGGAAA
4831  AGCGAGGAGG ATCGCGAGCA GCGACGAGGC CCGGCCCTCC CTCCGCTTCC AAAGAAACGC CCCCCATCGC
4901  CACTATATAC ATACCCCCCC CTCTCCTCCC ATCCCCCCAA CCCTACCACC ACCACCACCA CCACCTCCTC
4971  CCCCCTCGCT GCCGGACGAC GAGCTCCTCC CCCCTCCCCC TCCGCCGCCG CCGGTAACCA CCCCGCCCCT
5041  CTCCTCTTTC TTTCTCCGTT TTTTTTTTCG TCTCGGTCTC GATCTTTGGC CTTGGTAGTT TGGGTGGGCG
5111  AGAGCGGCTT CGTCGCCCAG ATCGGTGCGC GGGAGGGGCG GGATCTCGCG GCTGGCGTCT CCGGGCGTGA
              BamHI                             BglII
              ~~~~~~~~                          ~~~~~~~~
5181  GTCGGCCCGG ATCCTCGCGG GGAATGGGGC TCTCGGATGT AGATCTTCTT TCTTTCTTCT TTTTGTGGTA
5251  GAATTTGAAT CCCTCAGCAT TGTTCATCGG TAGTTTTTCT TTTCATGATT TGTGACAAAT GCAGCCTCGT
5321  GCGGAGCTTT TTTGTAGC
```

FIG._32E

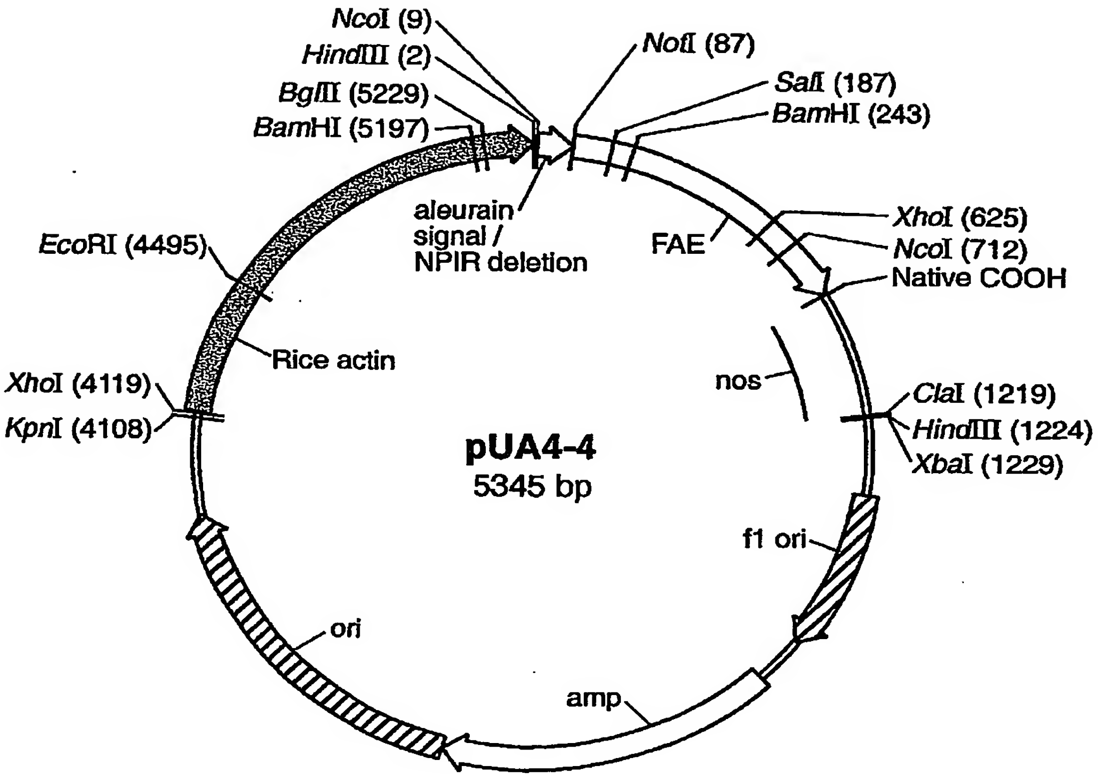

NcoI (9)
HindIII (2)
BglII (5229)
BamHI (5197)
NotI (87)
SalI (187)
BamHI (243)
aleurain signal / NPIR deletion
FAE
XhoI (625)
NcoI (712)
Native COOH
EcoRI (4495)
Rice actin
nos
ClaI (1219)
HindIII (1224)
XbaI (1229)
XhoI (4119)
KpnI (4108)
pUA4-4
5345 bp
f1 ori
ori
amp

FIG._33A

```
          NcoI
         ~~~~~~~
    HindIII
    ~~~~~~
               M  A  H  A   R  V  L   L  L  A   L  A  V  L   A  T  A   A  V  A   V
  1 AAGCTTACCA TGGCCCACGC CCGCGTCCTC CTCCTGGCGC TCGCCGTGCT GGCCACGGCC GCCGTCGCCG
                      NotI
                    ~~~~~~~~~~
    ·  A  S  S   R  A  A   A  S  T   Q  G  I  S   E  D  L   Y  S  R   L  V  E  M ·
 71 TCGCCTCCTC CCGCGCGGCC GCCTCCACGC AGGGCATCTC CGAAGACCTC TACAGCCGTT TAGTCGAAAT
                                                            SalI
                                                           ~~~~~~~~
    · A  T  I   S  Q  A   A  Y  A   D  L  C  N   I  P  S   T  I  I   K  G  E  K
141 GGCCACTATC TCCCAAGCTG CCTACGCCGA CCTGTGCAAC ATTCCGTCGA CTATTATCAA GGGAGAGAAA
                                        BamHI
                                        ~~~~~~~~
     I  Y  N   S  Q  T  D   I  N  G   W  I  L   R  D  D  S   S  K  E   I  I  T   V
211 ATTTACAATT CTCAAACTGA CATTAACGGA TGGATCCTCC GCGACGACAG CAGCAAAGAA ATAATCACCG
    ·  F  R  G   T  G  S   D  T  N   L  Q  L  D   T  N  Y   T  L  T   P  F  D  T ·
281 TCTTCCGTGG CACTGGTAGT GATACGAATC TACAACTCGA TACTAACTAC ACCCTCACGC CTTTCGACAC
    · L  P  Q   C  N  G   C  E  V   H  G  G  Y   Y  I  G   W  V  S   V  Q  D  Q
351 CCTACCACAA TGCAACGGTT GTGAAGTACA CGGTGGATAT TATATTGGAT GGGTCTCCGT CCAGGACCAA
     V  E  S   L  V  K  Q   Q  V  S   Q  Y  P   D  Y  A  L   T  V  T   G  H  X   L
421 GTCGAGTCGC TTGTCAAACA GCAGGTTAGC CAGTATCCGG ACTACGCGCT GACCGTGACC GGCCACKCCC
    ·  G  A  S   L  A  A   L  T  A   A  Q  L  S   A  T  Y   D  N  I   R  L  Y  T ·
491 TCGGCGCCTC CCTGGCGGCA CTCACTGCCG CCCAGCTGTC TGCGACATAC GACAACATCC GCCTGTACAC
                                                                            XhoI
                                                                          ~
    · F  G  E   P  R  S   G  N  Q   A  F  A  S   Y  M  N   D  A  F   Q  A  S  S
561 CTTCGGCGAA CCGCGCAGCG GCAATCAGGC CTTCGCGTCG TACATGAACG ATGCCTTCCA AGCCTCGAGC
     P  D  T   T  Q  Y  F   R  V  T   H  A  N   D  G  I  P   N  L  P   P  V  E   Q
631 CCAGATACGA CGCAGTATTT CCGGGTCACT CATGCCAACG ACGGCATCCC AAACCTGCCC CCGGTGGAGC
               NcoI
              ~~~~~~~
    ·  G  Y  A   H  G  G   V  E  Y   W  S  V  D   P  Y  S   A  Q  N   T  F  V  C ·
```

FIG._33B

```
  701  AGGGGTACGC CCATGGCGGT GTAGAGTACT GGAGCGTTGA TCCTTACAGC GCCCAGAACA CATTTGTCTG
       · T  G  D   E  V  Q   C  C  E  A   Q  G  G   Q  G  V   N  N  A  H   T  T  Y
  771  CACTGGGGAT GAAGTGCAGT GCTGTGAGGC CCAGGGCGGA CAGGGTGTGA ATAATGCGCA CACGACTTAT
        F  G  M   T  S  G  A   C  T  W   *
  841  TTTGGGATGA CGAGCGGAGC CTGTACATGG TGATCAGTCA TTTCAGCCTC CCCGAGTGTA CCAGGAAAGA
  911  TGGATGTCCT GGAGAGGGGG CCGCGTAACC ACTGAAGGAT GAGCTGTAAA GAAGCAGATC GTTCAAACAT
  981  TTGGCAATAA AGTTTCTTAA GATTGAATCC TGTTGCCGGT CTTGCGATGA TTATCATATA ATTTCTGTTG
 1051  AATTACGTTA AGCATGTAAT AATTAACATG TAATGCATGA CGTTATTTAT GAGATGGGTT TTTATGATTA
 1121  GAGTCCCGCA ATTATACATT TAATACGCGA TAGAAAACAA AATATAGCGC GCAAACTAGG ATAAATTATC
                                         HindIII
                                          ~~~~~~
                                   ClaI         XbaI
                                  ~~~~~~~      ~~~~~~~
 1191  GCGCGCGGTG TCATCTATGT TACTAGATCG ATAAGCTTCT AGAGCGGCCG GTGGAGCTCC AATTCGCCCT
 1261  ATAGTGAGTC GTATTACGCG CGCTCACTGG CCGTCGTTTT ACAACGTCGT GACTGGGAAA ACCCTGGCGT
 1331  TACCCAACTT AATCGCCTTG CAGCACATCC CCCTTTCGCC AGCTGGCGTA ATAGCGAAGA GGCCCGCACC
 1401  GATCGCCCTT CCCAACAGTT GCGCAGCCTG AATGGCGAAT GGGACGCGCC CTGTAGCGGC GCATTAAGCG
 1471  CGGCGGGTGT GGTGGTTACG CGCAGCGTGA CCGCTACACT TGCCAGCGCC CTAGCGCCCG CTCCTTTCGC
 1541  TTTCTTCCCT TCCTTTCTCG CCACGTTCGC CGGCTTTCCC CGTCAAGCTC TAAATCGGGG GCTCCCTTTA
 1611  GGGTTCCGAT TTAGTGCTTT ACGGCACCTC GACCCCAAAA AACTTGATTA GGGTGATGGT TCACGTAGTG
 1681  GGCCATCGCC CTGATAGACG GTTTTTCGCC CTTTGACGTT GGAGTCCACG TTCTTTAATA GTGGACTCTT
 1751  GTTCCAAACT GGAACAACAC TCAACCCTAT CTCGGTCTAT TCTTTTGATT TATAAGGGAT TTTGCCGATT
 1821  TCGGCCTATT GGTTAAAAAA TGAGCTGATT TAACAAAAAT TTAACGCGAA TTTTAACAAA ATATTAACGC
 1891  TTACAATTTA GGTGGCACTT TTCGGGGAAA TGTGCGCGGA ACCCCTATTT GTTTATTTTT CTAAATACAT
 1961  TCAAATATGT ATCCGCTCAT GAGACAATAA CCCTGATAAA TGCTTCAATA ATATTGAAAA AGGAAGAGTA
 2031  TGAGTATTCA ACATTTCCGT GTCGCCCTTA TTCCCTTTTT TGCGGCATTT TGCCTTCCTG TTTTTGCTCA
 2101  CCCAGAAACG CTGGTGAAAG TAAAAGATGC TGAAGATCAG TTGGGTGCAC GAGTGGGTTA CATCGAACTG
 2171  GATCTCAACA GCGGTAAGAT CCTTGAGAGT TTTCGCCCCG AAGAACGTTT TCCAATGATG AGCACTTTTA
 2241  AAGTTCTGCT ATGTGGCGCG GTATTATCCC GTATTGACGC CGGGCAAGAG CAACTCGGTC GCCGCATACA
 2311  CTATTCTCAG AATGACTTGG TTGAGTACTC ACCAGTCACA GAAAAGCATC TTACGGATGG CATGACAGTA
 2381  AGAGAATTAT GCAGTGCTGC CATAACCATG AGTGATAACA CTGCGGCCAA CTTACTTCTG ACAACGATCG
 2451  GAGGACCGAA GGAGCTAACC GCTTTTTTGC ACAACATGGG GGATCATGTA ACTCGCCTTG ATCGTTGGGA
 2521  ACCGGAGCTG AATGAAGCCA TACCAAACGA CGAGCGTGAC ACCACGATGC CTGTAGCAAT GGCAACAACG
 2591  TTGCGCAAAC TATTAACTGG CGAACTACTT ACTCTAGCTT CCCGGCAACA ATTAATAGAC TGGATGGAGG
 2661  CGGATAAAGT TGCAGGACCA CTTCTGCGCT CGGCCCTTCC GGCTGGCTGG TTTATTGCTG ATAAATCTGG
```

FIG._33C

```
2731  AGCCGGTGAG CGTGGGTCTC GCGGTATCAT TGCAGCACTG GGGCCAGATG GTAAGCCCTC CCGTATCGTA
2801  GTTATCTACA CGACGGGGAG TCAGGCAACT ATGGATGAAC GAAATAGACA GATCGCTGAG ATAGGTGCCT
2871  CACTGATTAA GCATTGGTAA CTGTCAGACC AAGTTTACTC ATATATACTT TAGATTGATT TAAAACTTCA
2941  TTTTTAATTT AAAAGGATCT AGGTGAAGAT CCTTTTTGAT AATCTCATGA CCAAAATCCC TTAACGTGAG
3011  TTTTCGTTCC ACTGAGCGTC AGACCCCGTA GAAAAGATCA AAGGATCTTC TTGAGATCCT TTTTTTCTGC
3081  GCGTAATCTG CTGCTTGCAA ACAAAAAAAC CACCGCTACC AGCGGTGGTT TGTTTGCCGG ATCAAGAGCT
3151  ACCAACTCTT TTTCCGAAGG TAACTGGCTT CAGCAGAGCG CAGATACCAA ATACTGTCCT TCTAGTGTAG
3221  CCGTAGTTAG GCCACCACTT CAAGAACTCT GTAGCACCGC CTACATACCT CGCTCTGCTA ATCCTGTTAC
3291  CAGTGGCTGC TGCCAGTGGC GATAAGTCGT GTCTTACCGG GTTGGACTCA AGACGATAGT TACCGGATAA
3361  GGCGCAGCGG TCGGGCTGAA CGGGGGGTTC GTGCACACAG CCCAGCTTGG AGCGAACGAC CTACACCGAA
3431  CTGAGATACC TACAGCGTGA GCTATGAGAA AGCGCCACGC TTCCCGAAGG GAGAAAGGCG GACAGGTATC
3501  CGGTAAGCGG CAGGGTCGGA ACAGGAGAGC GCACGAGGGA GCTTCCAGGG GGAAACGCCT GGTATCTTTA
3571  TAGTCCTGTC GGGTTTCGCC ACCTCTGACT TGAGCGTCGA TTTTTGTGAT GCTCGTCAGG GGGGCGGAGC
3641  CTATGGAAAA ACGCCAGCAA CGCGGCCTTT TTACGGTTCC TGGCCTTTTG CTGGCCTTTT GCTCACATGT
3711  TCTTTCCTGC GTTATCCCCT GATTCTGTGG ATAACCGTAT TACCGCCTTT GAGTGAGCTG ATACCGCTCG
3781  CCGCAGCCGA ACGACCGAGC GCAGCGAGTC AGTGAGCGAG GAAGCGGAAG AGCGCCCAAT ACGCAAACCG
3851  CCTCTCCCCG CGCGTTGGCC GATTCATTAA TGCAGCTGGC ACGACAGGTT TCCCGACTGG AAAGCGGGCA
3921  GTGAGCGCAA CGCAATTAAT GTGAGTTAGC TCACTCATTA GGCACCCCAG GCTTTACACT TTATGCTTCC
3991  GGCTCGTATG TTGTGTGGAA TTGTGAGCGG ATAACAATTT CACACAGGAA ACAGCTATGA CCATGATTAC
                                                    KpnI            XhoI
                                                  ~~~~~~~~        ~~~~~~~
4061  GCCAAGCGCG CAATTAACCC TCACTAAAGG GAACAAAAGC TGGGTACCGG GCCCCCCCTC GAGGTCATTC
4131  ATATGCTTGA GAAGAGAGTC GGGATAGTCC AAAATAAAAC AAAGGTAAGA TTACCTGGTC AAAAGTGAAA
4201  ACATCAGTTA AAAGGTGGTA TAAGTAAAAT ATCGGTAATA AAAGGTGGCC CAAAGTGAAA TTTACTCTTT
4271  TCTACTATTA TAAAAATTGA GGATGTTTTG TCGGTACTTT GATACGTCAT TTTTGTATGA ATTGGTTTTT
4341  AAGTTTATTC GCGATTTGGA AATGCATATC TGTATTTGAG TCGGTTTTTA AGTTCGTTGC TTTTGTAAAT
4411  ACAGAGGGAT TTGTATAAGA AATATCTTTA AAAAACCCAT ATGCTAATTT GACATAATTT TTGAGAAAAA
                   EcoRI
                  ~~~~~~~~
4481  TATATATTCA GGCGAATTCC ACAATGAACA ATAATAAGAT TAAAATAGCT TGCCCCCGTT GCAGCGATGG
4551  GTATTTTTTC TAGTAAAATA AAAGATAAAC TTAGACTCAA AACATTTACA AAAACAACCC CTAAAGTCCT
4621  AAAGCCCAAA GTGCTATGCA CGATCCATAG CAAGCCCAGC CCAACCCAAC CCAACCCAAC CCACCCCAGT
4691  GCAGCCAACT GGCAAATAGT CTCCACCCCC GGCACTATCA CCGTGAGTTG TCCGCACCAC CGCACGTCTC
4761  GCAGCCAAAA AAAAAAAAAG AAAGAAAAAA AAGAAAAAGA AAAACAGCAG GTGGGTCCGG GTCGTGGGGG
4831  CCGGAAAAGC GAGGAGGATC GCGAGCAGCG ACGAGGCCCG GCCCTCCCTC CGCTTCCAAA GAAACGCCCC
```

FIG._33D

```
4901  CCATCGCCAC TATATACATA CCCCCCCCTC TCCTCCCATC CCCCCAACCC TACCACCACC ACCACCACCA
4971  CCTCCTCCCC CCTCGCTGCC GGACGACGAG CTCCTCCCCC CTCCCCCTCC GCCGCCGCCG GTAACCACCC
5041  CGCCCCTCTC CTCTTTCTTT CTCCGTTTTT TTTTTCGTCT CGGTCTCGAT CTTTGGCCTT GGTAGTTTGG
5111  GTGGGCGAGA GCGGCTTCGT CGCCCAGATC GGTGCGCGGG AGGGGCGGGA TCTCGCGGCT GGCGTCTCCG
                      BamHI                              BglII
                      ~~~~~~~                            ~~~~~~
5181  GGCGTGAGTC GGCCCGGATC CTCGCGGGGA ATGGGGCTCT CGGATGTAGA TCTTCTTTCT TTCTTCTTTT
5251  TGTGGTAGAA TTTGAATCCC TCAGCATTGT TCATCGGTAG TTTTTCTTTT CATGATTTGT GACAAATGCA
5321  GCCTCGTGCG GAGCTTTTTT GTAGC
```

FIG._33E

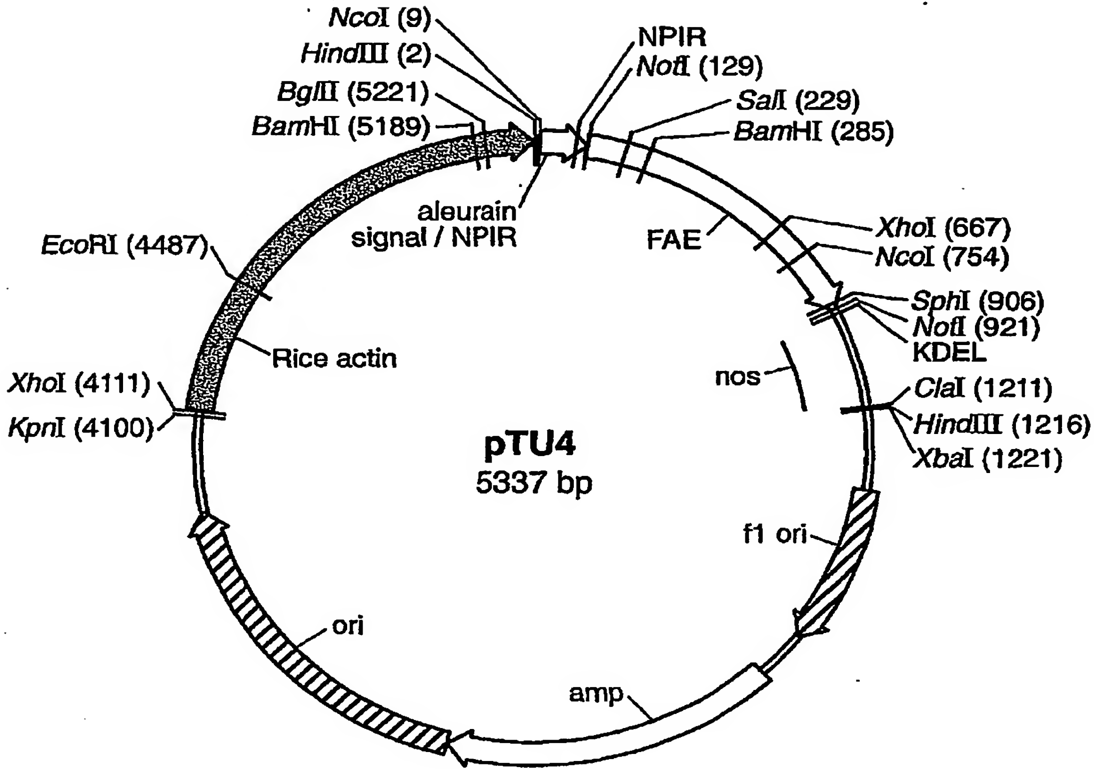

NcoI (9)
HindIII (2)
BglII (5221)
BamHI (5189)
NPIR
NotI (129)
SalI (229)
BamHI (285)
aleurain signal / NPIR
FAE
XhoI (667)
NcoI (754)
EcoRI (4487)
SphI (906)
NotI (921)
KDEL
Rice actin
nos
ClaI (1211)
HindIII (1216)
XbaI (1221)
XhoI (4111)
KpnI (4100)
pTU4
5337 bp
f1 ori
ori
amp

FIG._34A

```
            NcoI
           ~~~~~~~~
    HindIII
    ~~~~~~~
               M  A  H  A   R  V  L   L  L  A   L  A  V  L   A  T  A   A  V  A   V
  1 AAGCTTACCA TGGCCCACGC CCGCGTCCTC CTCCTGGCGC TCGCCGTGCT GGCCACGGCC GCCGTCGCCG
                                                                   NotI
                                                                 ~~~~~~~~~
    •  A  S  S   S  S  F   A  D  S   N  P  I  R   P  V  T  D  R  A    A  A  S  T •
 71 TCGCCTCCTC CTCCTCCTTC GCCGACTCCA ACCCGATCCG GCCCGTCACC GACCGCGCGG CCGCCTCCAC
    • Q  G  I   S  E  D   L  Y  S  R   L  V  E   M  A  T   I  S  Q  A   A  Y  A
141 GCAGGGCATC TCCGAAGACC TCTACAGCCG TTTAGTCGAA ATGGCCACTA TCTCCCAAGC TGCCTACGCC
                       SalI
                      ~~~~~~~~
     D  L  C   N  I  P  S   T  I  I   K  G  E   K  I  Y  N   S  Q  T   D  I  N   G
211 GACCTGTGCA ACATTCCGTC GACTATTATC AAGGGAGAGA AAATTTACAA TTCTCAAACT GACATTAACG
       BamHI
       ~~~~~~~
    •  W  I  L   R  D  D   S  S  K   E  I  I  T   V  F  R  G  T  G    S  D  T  N •
281 GATGGATCCT CCGCGACGAC AGCAGCAAAG AAATAATCAC CGTCTTCCGT GGCACTGGTA GTGATACGAA
    • L  Q  L   D  T  N   Y  T  L  T   P  F  D   T  L  P   Q  C  N  G   C  E  V
351 TCTACAACTC GATACTAACT ACACCCTCAC GCCTTTCGAC ACCCTACCAC AATGCAACGG TTGTGAAGTA
     H  G  G   Y  Y  I  G   W  V  S   V  Q  D   Q  V  E  S   L  V  K   Q  Q  V   S
421 CACGGTGGAT ATTATATTGG ATGGGTCTCC GTCCAGGACC AAGTCGAGTC GCTTGTCAAA CAGCAGGTTA
    •  Q  Y  P   D  Y  A   L  T  V   T  G  H  X   L  G  A  S  L  A    A  L  T  A •
491 GCCAGTATCC GGACTACGCG CTGACCGTGA CCGGCCACKC CCTCGGCGCC TCCCTGGCGG CACTCACTGC
    • A  Q  L   S  A  T   Y  D  N  I   R  L  Y   T  F  G   E  P  R  S   G  N  Q
561 CGCCCAGCTG TCTGCGACAT ACGACAACAT CCGCCTGTAC ACCTTCGGCG AACCGCGCAG CGGCAATCAG
                                           XhoI
                                          ~~~~~~~~
     A  F  A   S  Y  M  N   D  A  F   Q  A  S   S  P  D  T   T  Q  Y   F  R  V   T
631 GCCTTCGCGT CGTACATGAA CGATGCCTTC CAAGCCTCGA GCCCAGATAC GACGCAGTAT TTCCGGGTCA
                                                              NcoI
                                                             ~~~~~~~~
    •  H  A  N   D  G  I   P  N  L   P  P  V  E   Q  G  Y  A  H  G    G  V  E  Y •
```

FIG._34B

```
 701  CTCATGCCAA CGACGGCATC CCAAACCTGC CCCCGGTGGA GCAGGGGTAC GCCCATGGCG GTGTAGAGTA
      · W  S  V   D  P  Y  S   A  Q  N   T  F  V   C  T  G  D   E  V  Q   C  C  E
 771  CTGGAGCGTT GATCCTTACA GCGCCCAGAA CACATTTGTC TGCACTGGGG ATGAAGTGCA GTGCTGTGAG
                                                                         SphI
                                                                        ~~~~
       A  Q  G  G   Q  G  V   N  N  A   H  T  T  Y   F  G  M   T  S  G   A  C  T  W
 841  GCCCAGGGCG GACAGGGTGT GAATAATGCG CACACGACTT ATTTTGGGAT GACGAGCGGC GCATGCACCT
                NotI
              ~~~~~~~~~~
      ·  P  V  A   A  A  E   P  L  K  D   E  L  *
 911  GGCCGGTCGC GGCCGCGGAA CCACTGAAGG ATGAGCTGTA AAGAAGCAGA TCGTTCAAAC ATTTGGCAAT
 981  AAAGTTTCTT AAGATTGAAT CCTGTTGCCG GTCTTGCGAT GATTATCATA TAATTTCTGT TGAATTACGT
1051  TAAGCATGTA ATAATTAACA TGTAATGCAT GACGTTATTT ATGAGATGGG TTTTTATGAT TAGAGTCCCG
1121  CAATTATACA TTTAATACGC GATAGAAAAC AAAATATAGC GCGCAAACTA GGATAAATTA TCGCGCGCGG
                                HindIII
                                ~~~~~~~~
                          ClaI        XbaI
                         ~~~~~~~~    ~~~~~~~
1191  TGTCATCTAT GTTACTAGAT CGATAAGCTT CTAGAGCGGC CGGTGGAGCT CCAATTCGCC CTATAGTGAG
1261  TCGTATTACG CGCGCTCACT GGCCGTCGTT TTACAACGTC GTGACTGGGA AAACCCTGGC GTTACCCAAC
1331  TTAATCGCCT TGCAGCACAT CCCCCTTTCG CCAGCTGGCG TAATAGCGAA GAGGCCCGCA CCGATCGCCC
1401  TTCCCAACAG TTGCGCAGCC TGAATGGCGA ATGGGACGCG CCCTGTAGCG GCGCATTAAG CGCGGCGGGT
1471  GTGGTGGTTA CGCGCAGCGT GACCGCTACA CTTGCCAGCG CCCTAGCGCC CGCTCCTTTC GCTTTCTTCC
1541  CTTCCTTTCT CGCCACGTTC GCCGGCTTTC CCCGTCAAGC TCTAAATCGG GGGCTCCCTT TAGGGTTCCG
1611  ATTTAGTGCT TTACGGCACC TCGACCCCAA AAAACTTGAT TAGGGTGATG GTTCACGTAG TGGGCCATCG
1681  CCCTGATAGA CGGTTTTTCG CCCTTTGACG TTGGAGTCCA CGTTCTTTAA TAGTGGACTC TTGTTCCAAA
1751  CTGGAACAAC ACTCAACCCT ATCTCGGTCT ATTCTTTTGA TTTATAAGGG ATTTTGCCGA TTTCGGCCTA
1821  TTGGTTAAAA AATGAGCTGA TTTAACAAAA ATTTAACGCG AATTTTAACA AAATATTAAC GCTTACAATT
1891  TAGGTGGCAC TTTTCGGGGA AATGTGCGCG GAACCCCTAT TTGTTTATTT TTCTAAATAC ATTCAAATAT
1961  GTATCCGCTC ATGAGACAAT AACCCTGATA AATGCTTCAA TAATATTGAA AAAGGAAGAG TATGAGTATT
2031  CAACATTTCC GTGTCGCCCT TATTCCCTTT TTTGCGGCAT TTTGCCTTCC TGTTTTTGCT CACCCAGAAA
2101  CGCTGGTGAA AGTAAAAGAT GCTGAAGATC AGTTGGGTGC ACGAGTGGGT TACATCGAAC TGGATCTCAA
2171  CAGCGGTAAG ATCCTTGAGA GTTTTCGCCC CGAAGAACGT TTTCCAATGA TGAGCACTTT TAAAGTTCTG
2241  CTATGTGGCG CGGTATTATC CCGTATTGAC GCCGGGCAAG AGCAACTCGG TCGCCGCATA CACTATTCTC
2311  AGAATGACTT GGTTGAGTAC TCACCAGTCA CAGAAAAGCA TCTTACGGAT GGCATGACAG TAAGAGAATT
2381  ATGCAGTGCT GCCATAACCA TGAGTGATAA CACTGCGGCC AACTTACTTC TGACAACGAT CGGAGGACCG
```

FIG._34C

```
2451  AAGGAGCTAA CCGCTTTTTT GCACAACATG GGGGATCATG TAACTCGCCT TGATCGTTGG GAACCGGAGC
2521  TGAATGAAGC CATACCAAAC GACGAGCGTG ACACCACGAT GCCTGTAGCA ATGGCAACAA CGTTGCGCAA
2591  ACTATTAACT GGCGAACTAC TTACTCTAGC TTCCCGGCAA CAATTAATAG ACTGGATGGA GGCGGATAAA
2661  GTTGCAGGAC CACTTCTGCG CTCGGCCCTT CCGGCTGGCT GGTTTATTGC TGATAAATCT GGAGCCGGTG
2731  AGCGTGGGTC TCGCGGTATC ATTGCAGCAC TGGGGCCAGA TGGTAAGCCC TCCCGTATCG TAGTTATCTA
2801  CACGACGGGG AGTCAGGCAA CTATGGATGA ACGAAATAGA CAGATCGCTG AGATAGGTGC CTCACTGATT
2871  AAGCATTGGT AACTGTCAGA CCAAGTTTAC TCATATATAC TTTAGATTGA TTTAAAACTT CATTTTTAAT
2941  TTAAAAGGAT CTAGGTGAAG ATCCTTTTTG ATAATCTCAT GACCAAAATC CCTTAACGTG AGTTTTCGTT
3011  CCACTGAGCG TCAGACCCCG TAGAAAAGAT CAAAGGATCT TCTTGAGATC CTTTTTTTCT GCGCGTAATC
3081  TGCTGCTTGC AAACAAAAAA ACCACCGCTA CCAGCGGTGG TTTGTTTGCC GGATCAAGAG CTACCAACTC
3151  TTTTTCCGAA GGTAACTGGC TTCAGCAGAG CGCAGATACC AAATACTGTC CTTCTAGTGT AGCCGTAGTT
3221  AGGCCACCAC TTCAAGAACT CTGTAGCACC GCCTACATAC CTCGCTCTGC TAATCCTGTT ACCAGTGGCT
3291  GCTGCCAGTG GCGATAAGTC GTGTCTTACC GGGTTGGACT CAAGACGATA GTTACCGGAT AAGGCGCAGC
3361  GGTCGGGCTG AACGGGGGGT TCGTGCACAC AGCCCAGCTT GGAGCGAACG ACCTACACCG AACTGAGATA
3431  CCTACAGCGT GAGCTATGAG AAAGCGCCAC GCTTCCCGAA GGGAGAAAGG CGGACAGGTA TCCGGTAAGC
3501  GGCAGGGTCG GAACAGGAGA GCGCACGAGG GAGCTTCCAG GGGGAAACGC CTGGTATCTT TATAGTCCTG
3571  TCGGGTTTCG CCACCTCTGA CTTGAGCGTC GATTTTTGTG ATGCTCGTCA GGGGGGCGGA GCCTATGGAA
3641  AAACGCCAGC AACGCGGCCT TTTTACGGTT CCTGGCCTTT TGCTGGCCTT TTGCTCACAT GTTCTTTCCT
3711  GCGTTATCCC CTGATTCTGT GGATAACCGT ATTACCGCCT TTGAGTGAGC TGATACCGCT CGCCGCAGCC
3781  GAACGACCGA GCGCAGCGAG TCAGTGAGCG AGGAAGCGGA AGAGCGCCCA ATACGCAAAC CGCCTCTCCC
3851  CGCGCGTTGG CCGATTCATT AATGCAGCTG GCACGACAGG TTTCCCGACT GGAAAGCGGG CAGTGAGCGC
3921  AACGCAATTA ATGTGAGTTA GCTCACTCAT TAGGCACCCC AGGCTTTACA CTTTATGCTT CCGGCTCGTA
3991  TGTTGTGTGG AATTGTGAGC GGATAACAAT TTCACACAGG AAACAGCTAT GACCATGATT ACGCCAAGCG
                                                 KpnI                XhoI
                                              ~~~~~~~~            ~~~~~~
4061  CGCAATTAAC CCTCACTAAA GGGAACAAAA GCTGGGTACC GGGCCCCCCC TCGAGGTCAT TCATATGCTT
4131  GAGAAGAGAG TCGGGATAGT CCAAAATAAA ACAAAGGTAA GATTACCTGG TCAAAAGTGA AAACATCAGT
4201  TAAAAGGTGG TATAAGTAAA ATATCGGTAA TAAAAGGTGG CCCAAAGTGA AATTTACTCT TTTCTACTAT
4271  TATAAAAATT GAGGATGTTT TGTCGGTACT TTGATACGTC ATTTTTGTAT GAATTGGTTT TTAAGTTTAT
4341  TCGCGATTTG GAAATGCATA TCTGTATTTG AGTCGGTTTT TAAGTTCGTT GCTTTTGTAA ATACAGAGGG
4411  ATTTGTATAA GAAATATCTT TAAAAAACCC ATATGCTAAT TTGACATAAT TTTTGAGAAA AATATATATT
           EcoRI
          ~~~~~~~~
4481  CAGGCGAATT CCACAATGAA CAATAATAAG ATTAAAATAG CTTGCCCCCG TTGCAGCGAT GGGTATTTTT
4551  TCTAGTAAAA TAAAAGATAA ACTTAGACTC AAAACATTTA CAAAAACAAC CCCTAAAGTC CTAAAGCCCA
```

FIG._34D

```
4621  AAGTGCTATG CACGATCCAT AGCAAGCCCA GCCCAACCCA ACCCAACCCA ACCCACCCCA GTGCAGCCAA
4691  CTGGCAAATA GTCTCCACCC CCGGCACTAT CACCGTGAGT TGTCCGCACC ACCGCACGTC TCGCAGCCAA
4761  AAAAAAAAAA AGAAAGAAAA AAAAGAAAAA GAAAAACAGC AGGTGGGTCC GGGTCGTGGG GGCCGGAAAA
4831  GCGAGGAGGA TCGCGAGCAG CGACGAGGCC CGGCCCTCCC TCCGCTTCCA AAGAAACGCC CCCCATCGCC
4901  ACTATATACA TACCCCCCCC TCTCCTCCCA TCCCCCCAAC CCTACCACCA CCACCACCAC CACCTCCTCC
4971  CCCCTCGCTG CCGGACGACG AGCTCCTCCC CCCTCCCCCT CCGCCGCCGC CGGTAACCAC CCCGCCCCTC
5041  TCCTCTTTCT TTCTCCGTTT TTTTTTTCGT CTCGGTCTCG ATCTTTGGCC TTGGTAGTTT GGGTGGGCGA
5111  GAGCGGCTTC GTCGCCCAGA TCGGTGCGCG GGAGGGGCGG GATCTCGCGG CTGGCGTCTC CGGGCGTGAG
             BamHI                              BglII
             ~~~~~~~~                           ~~~~~~~
5181  TCGGCCCGGA TCCTCGCGGG GAATGGGGCT CTCGGATGTA GATCTTCTTT CTTTCTTCTT TTTGTGGTAG
5251  AATTTGAATC CCTCAGCATT GTTCATCGGT AGTTTTTCTT TTCATGATTT GTGACAAATG CAGCCTCGTG
5321  CGGAGCTTTT TTGTAGC
```

FIG._34E

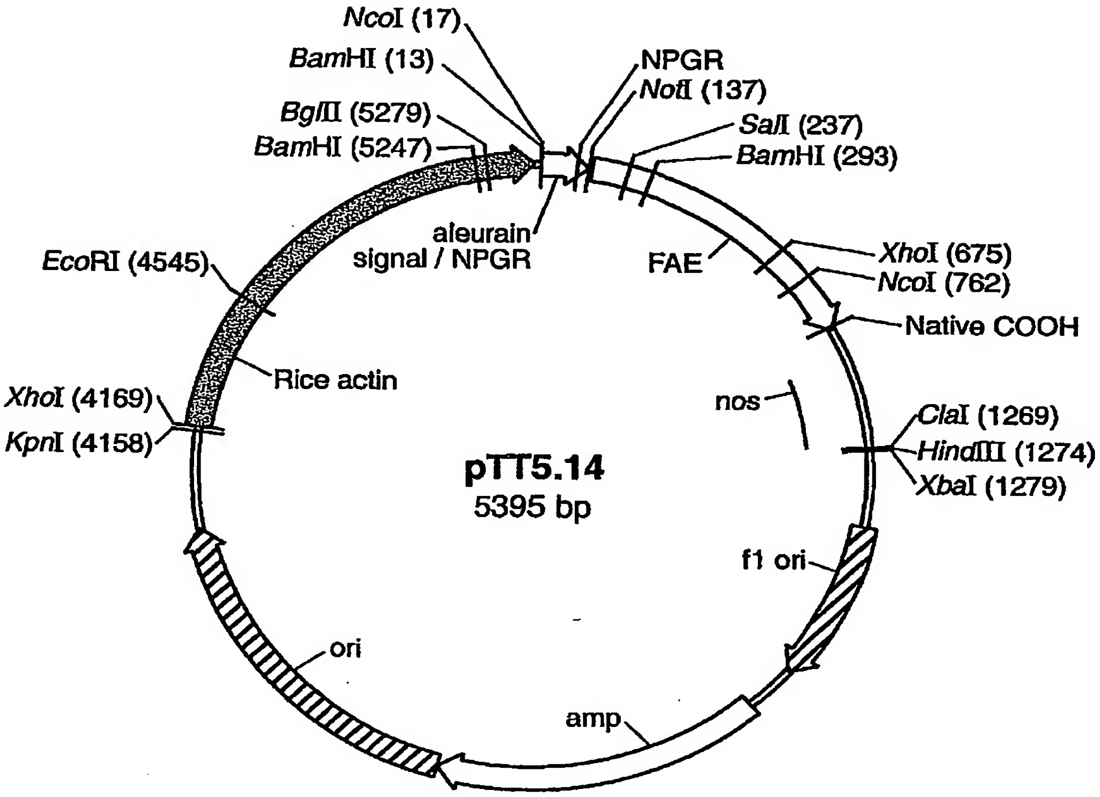

NcoI (17)
BamHI (13)
BglII (5279)
BamHI (5247)
NPGR
NotI (137)
SalI (237)
BamHI (293)
aleurain
signal / NPGR
FAE
XhoI (675)
NcoI (762)
Native COOH
EcoRI (4545)
Rice actin
XhoI (4169)
KpnI (4158)
nos
ClaI (1269)
HindIII (1274)
XbaI (1279)
pTT5.14
5395 bp
f1 ori
ori
amp

FIG._35A

```
                     NcoI
                    ~~~~~~~
                BamHI
                ~~~~~~
                       M   A  H  A   R  V  L  L   L  A  L   A  V  L   A  T  A  A·
  1 CCTGACGCCG AGGATCCATG GCCCACGCCC GCGTCCTCCT CCTGGCGCTC GCCGTGCTGG CCACGGCCGC
                                                                           NotI
    · V  A  V   A  S  S   S  S  F  A   D  S  N   P  G  R   P  V  T  D   R  A  A
 71 CGTCGCCGTC GCCTCCTCCT CCTCCTTCGC CGACTCCAAC CCGGGCCGGC CCGTCACCGA CCGCGCGGCC
    NotI
    ~~
     A  S  T   Q  G  I  S   E  D  L   Y  S  R   L  V  E  M   A  T  I   S  Q  A   A
141 GCCTCCACGC AGGGCATCTC CGAAGACCTC TACAGCCGTT TAGTCGAAAT GGCCACTATC TCCCAAGCTG
                                SalI
                               ~~~~~~~~
     · Y  A  D   L  C  N   I  P  S   T  I  I  K   G  E  K   I  Y  N   S  Q  T  D·
211 CCTACGCCGA CCTGTGCAAC ATTCCGTCGA CTATTATCAA GGGAGAGAAA ATTTACAATT CTCAAACTGA
                BamHI
                ~~~~~~~
    · I  N  G   W  I  L   R  D  D  S   S  K  E   I  I  T   V  F  R  G   T  G  S
281 CATTAACGGA TGGATCCTCC GCGACGACAG CAGCAAAGAA ATAATCACCG TCTTCCGTGG CACTGGTAGT
     D  T  N   L  Q  L  D   T  N  Y   T  L  T   P  F  D  T   L  P  Q   C  N  G   C
351 GATACGAATC TACAACTCGA TACTAACTAC ACCCTCACGC CTTTCGACAC CCTACCACAA TGCAACGGTT
     · E  V  H   G  G  Y   Y  I  G   W  V  S  V   Q  D  Q   V  E  S   L  V  K  Q·
421 GTGAAGTACA CGGTGGATAT TATATTGGAT GGGTCTCCGT CCAGGACCAA GTCGAGTCGC TTGTCAAACA
    · Q  V  S   Q  Y  P   D  Y  A  L   T  V  T   G  H  X   L  G  A  S   L  A  A
491 GCAGGTTAGC CAGTATCCGG ACTACGCGCT GACCGTGACC GGCCACKCCC TCGGCGCCTC CCTGGCGGCA
     L  T  A   A  Q  L  S   A  T  Y   D  N  I   R  L  Y  T   F  G  E   P  R  S   G
561 CTCACTGCCG CCCAGCTGTC TGCGACATAC GACAACATCC GCCTGTACAC CTTCGGCGAA CCGCGCAGCG
                                                    XhoI
                                                   ~~~~~~~~
     · N  Q  A   F  A  S   Y  M  N   D  A  F  Q   A  S  S   P  D  T   T  Q  Y  F·
631 GCAATCAGGC CTTCGCGTCG TACATGAACG ATGCCTTCCA AGCCTCGAGC CCAGATACGA CGCAGTATTT
                                                                       NcoI
                                                                      ~~~~
    · R  V  T   H  A  N   D  G  I  P   N  L  P   P  V  E   Q  G  Y  A   H  G  G
```

FIG._35B

```
 701  CCGGGTCACT CATGCCAACG ACGGCATCCC AAACCTGCCC CCGGTGGAGC AGGGGTACGC CCATGGCGGT
       V  E  Y  W   S  V  D   P  Y  S   A  Q  N  T   F  V  C   T  G  D   E  V  Q  C
 771  GTAGAGTACT GGAGCGTTGA TCCTTACAGC GCCCAGAACA CATTTGTCTG CACTGGGGAT GAAGTGCAGT
      ·  C  E  A   Q  G  G   Q  G  V  N   N  A  H   T  T  Y   F  G  M  T   S  G  A ·
 841  GCTGTGAGGC CCAGGGCGGA CAGGGTGTGA ATAATGCGCA CACGACTTAT TTTGGGATGA CGAGCGGAGC
      · C  T  W   *
 911  CTGTACATGG TGATCAGTCA TTTCAGCCTC CCCGAGTGTA CCAGGAAAGA TGGATGTCCT GGAGAGGGGG
 981  CCGCGTAACC ACTGAAGGAT GAGCTGTAAA GAAGCAGATC GTTCAAACAT TTGGCAATAA AGTTTCTTAA
1051  GATTGAATCC TGTTGCCGGT CTTGCGATGA TTATCATATA ATTTCTGTTG AATTACGTTA AGCATGTAAT
1121  AATTAACATG TAATGCATGA CGTTATTTAT GAGATGGGTT TTTATGATTA GAGTCCCGCA ATTATACATT
1191  TAATACGCGA TAGAAAACAA AATATAGCGC GCAAACTAGG ATAAATTATC GCGCGCGGTG TCATCTATGT
                   HindIII
                   ~~~~~~
             ClaI        XbaI
            ~~~~~~~     ~~~~~~~
1261  TACTAGATCG ATAAGCTTCT AGAGCGGCCG GTGGAGCTCC AATTCGCCCT ATAGTGAGTC GTATTACGCG
1331  CGCTCACTGG CCGTCGTTTT ACAACGTCGT GACTGGGAAA ACCCTGGCGT TACCCAACTT AATCGCCTTG
1401  CAGCACATCC CCCTTTCGCC AGCTGGCGTA ATAGCGAAGA GGCCCGCACC GATCGCCCTT CCCAACAGTT
1471  GCGCAGCCTG AATGGCGAAT GGGACGCGCC CTGTAGCGGC GCATTAAGCG CGGCGGGTGT GGTGGTTACG
1541  CGCAGCGTGA CCGCTACACT TGCCAGCGCC CTAGCGCCCG CTCCTTTCGC TTTCTTCCCT TCCTTTCTCG
1611  CCACGTTCGC CGGCTTTCCC CGTCAAGCTC TAAATCGGGG GCTCCCTTTA GGGTTCCGAT TTAGTGCTTT
1681  ACGGCACCTC GACCCCAAAA AACTTGATTA GGGTGATGGT TCACGTAGTG GGCCATCGCC CTGATAGACG
1751  GTTTTTCGCC CTTTGACGTT GGAGTCCACG TTCTTTAATA GTGGACTCTT GTTCCAAACT GGAACAACAC
1821  TCAACCCTAT CTCGGTCTAT TCTTTTGATT TATAAGGGAT TTTGCCGATT TCGGCCTATT GGTTAAAAAA
1891  TGAGCTGATT TAACAAAAAT TTAACGCGAA TTTTAACAAA ATATTAACGC TTACAATTTA GGTGGCACTT
1961  TTCGGGGAAA TGTGCGCGGA ACCCCTATTT GTTTATTTTT CTAAATACAT TCAAATATGT ATCCGCTCAT
2031  GAGACAATAA CCCTGATAAA TGCTTCAATA ATATTGAAAA AGGAAGAGTA TGAGTATTCA ACATTTCCGT
2101  GTCGCCCTTA TTCCCTTTTT TGCGGCATTT TGCCTTCCTG TTTTTGCTCA CCCAGAAACG CTGGTGAAAG
2171  TAAAAGATGC TGAAGATCAG TTGGGTGCAC GAGTGGGTTA CATCGAACTG GATCTCAACA GCGGTAAGAT
2241  CCTTGAGAGT TTTCGCCCCG AAGAACGTTT TCCAATGATG AGCACTTTTA AAGTTCTGCT ATGTGGCGCG
2311  GTATTATCCC GTATTGACGC CGGGCAAGAG CAACTCGGTC GCCGCATACA CTATTCTCAG AATGACTTGG
2381  TTGAGTACTC ACCAGTCACA GAAAAGCATC TTACGGATGG CATGACAGTA AGAGAATTAT GCAGTGCTGC
2451  CATAACCATG AGTGATAACA CTGCGGCCAA CTTACTTCTG ACAACGATCG GAGGACCGAA GGAGCTAACC
2521  GCTTTTTTGC ACAACATGGG GGATCATGTA ACTCGCCTTG ATCGTTGGGA ACCGGAGCTG AATGAAGCCA
2591  TACCAAACGA CGAGCGTGAC ACCACGATGC CTGTAGCAAT GGCAACAACG TTGCGCAAAC TATTAACTGG
2661  CGAACTACTT ACTCTAGCTT CCCGGCAACA ATTAATAGAC TGGATGGAGG CGGATAAAGT TGCAGGACCA
```

FIG._35C

```
2731  CTTCTGCGCT CGGCCCTTCC GGCTGGCTGG TTTATTGCTG ATAAATCTGG AGCCGGTGAG CGTGGGTCTC
2801  GCGGTATCAT TGCAGCACTG GGGCCAGATG GTAAGCCCTC CCGTATCGTA GTTATCTACA CGACGGGGAG
2871  TCAGGCAACT ATGGATGAAC GAAATAGACA GATCGCTGAG ATAGGTGCCT CACTGATTAA GCATTGGTAA
2941  CTGTCAGACC AAGTTTACTC ATATATACTT TAGATTGATT TAAAACTTCA TTTTTAATTT AAAAGGATCT
3011  AGGTGAAGAT CCTTTTTGAT AATCTCATGA CCAAAATCCC TTAACGTGAG TTTTCGTTCC ACTGAGCGTC
3081  AGACCCCGTA GAAAAGATCA AAGGATCTTC TTGAGATCCT TTTTTTCTGC GCGTAATCTG CTGCTTGCAA
3151  ACAAAAAAAC CACCGCTACC AGCGGTGGTT TGTTTGCCGG ATCAAGAGCT ACCAACTCTT TTTCCGAAGG
3221  TAACTGGCTT CAGCAGAGCG CAGATACCAA ATACTGTCCT TCTAGTGTAG CCGTAGTTAG GCCACCACTT
3291  CAAGAACTCT GTAGCACCGC CTACATACCT CGCTCTGCTA ATCCTGTTAC CAGTGGCTGC TGCCAGTGGC
3361  GATAAGTCGT GTCTTACCGG GTTGGACTCA AGACGATAGT TACCGGATAA GGCGCAGCGG TCGGGCTGAA
3431  CGGGGGGTTC GTGCACACAG CCCAGCTTGG AGCGAACGAC CTACACCGAA CTGAGATACC TACAGCGTGA
3501  GCTATGAGAA AGCGCCACGC TTCCCGAAGG GAGAAAGGCG GACAGGTATC CGGTAAGCGG CAGGGTCGGA
3571  ACAGGAGAGC GCACGAGGGA GCTTCCAGGG GGAAACGCCT GGTATCTTTA TAGTCCTGTC GGGTTTCGCC
3641  ACCTCTGACT TGAGCGTCGA TTTTTGTGAT GCTCGTCAGG GGGGCGGAGC CTATGGAAAA ACGCCAGCAA
3711  CGCGGCCTTT TTACGGTTCC TGGCCTTTTG CTGGCCTTTT GCTCACATGT TCTTTCCTGC GTTATCCCCT
3781  GATTCTGTGG ATAACCGTAT TACCGCCTTT GAGTGAGCTG ATACCGCTCG CCGCAGCCGA ACGACCGAGC
3851  GCAGCGAGTC AGTGAGCGAG GAAGCGGAAG AGCGCCCAAT ACGCAAACCG CCTCTCCCCG CGCGTTGGCC
3921  GATTCATTAA TGCAGCTGGC ACGACAGGTT TCCCGACTGG AAAGCGGGCA GTGAGCGCAA CGCAATTAAT
3991  GTGAGTTAGC TCACTCATTA GGCACCCCAG GCTTTACACT TTATGCTTCC GGCTCGTATG TTGTGTGGAA
4061  TTGTGAGCGG ATAACAATTT CACACAGGAA ACAGCTATGA CCATGATTAC GCCAAGCGCG CAATTAACCC
                               KpnI             XhoI
                              ~~~~~~~          ~~~~~~
4131  TCACTAAAGG GAACAAAAGC TGGGTACCGG GCCCCCCCTC GAGGTCATTC ATATGCTTGA GAAGAGAGTC
4201  GGGATAGTCC AAAATAAAAC AAAGGTAAGA TTACCTGGTC AAAAGTGAAA ACATCAGTTA AAAGGTGGTA
4271  TAAGTAAAAT ATCGGTAATA AAAGGTGGCC CAAAGTGAAA TTTACTCTTT TCTACTATTA TAAAAATTGA
4341  GGATGTTTTG TCGGTACTTT GATACGTCAT TTTTGTATGA ATTGGTTTTT AAGTTTATTC GCGATTTGGA
4411  AATGCATATC TGTATTTGAG TCGGTTTTTA AGTTCGTTGC TTTTGTAAAT ACAGAGGGAT TTGTATAAGA
                                                                           EcoRI
                                                                           ~
4481  AATATCTTTA AAAAACCCAT ATGCTAATTT GACATAATTT TTGAGAAAAA TATATATTCA GGCGAATTCC
4551  ACAATGAACA ATAATAAGAT TAAAATAGCT TGCCCCCGTT GCAGCGATGG GTATTTTTTC TAGTAAAATA
4621  AAAGATAAAC TTAGACTCAA AACATTTACA AAAACAACCC CTAAAGTCCT AAAGCCCAAA GTGCTATGCA
4691  CGATCCATAG CAAGCCCAGC CCAACCCAAC CCAACCCAAC CCACCCCAGT GCAGCCAACT GGCAAATAGT
4761  CTCCACCCCC GGCACTATCA CCGTGAGTTG TCCGCACCAC CGCACGTCTC GCAGCCAAAA AAAAAAAAAG
4831  AAAGAAAAAA AAGAAAAAGA AAAACAGCAG GTGGGTCCGG GTCGTGGGGG CCGGAAAAGC GAGGAGGATC
```

FIG._35D

```
4901  GCGAGCAGCG ACGAGGCCCG GCCCTCCCTC CGCTTCCAAA GAAACGCCCC CCATCGCCAC TATATACATA
4971  CCCCCCCCTC TCCTCCCATC CCCCCAACCC TACCACCACC ACCACCACCA CCTCCTCCCC CCTCGCTGCC
5041  GGACGACGAG CTCCTCCCCC CTCCCCCTCC GCCGCCGCCG GTAACCACCC CGCCCCTCTC CTCTTTCTTT
5111  CTCCGTTTTT TTTTTCGTCT CGGTCTCGAT CTTTGGCCTT GGTAGTTTGG GTGGGCGAGA GCGGCTTCGT
                                                                           BamHI
5181  CGCCCAGATC GGTGCGCGGG AGGGGCGGGA TCTCGCGGCT GGCGTCTCCG GGCGTGAGTC GGCCCGGATC
      BamHI                    BglII
      ~                        ~~~~~~~~
5251  CTCGCGGGGA ATGGGGCTCT CGGATGTAGA TCTTCTTTCT TTCTTCTTTT TGTGGTAGAA TTTGAATCCC
5321  TCAGCATTGT TCATCGGTAG TTTTTCTTTT CATGATTTGT GACAAATGCA GCCTCGTGCG GAGCTTTTTT
5391  GTAGC
```

FIG._35E

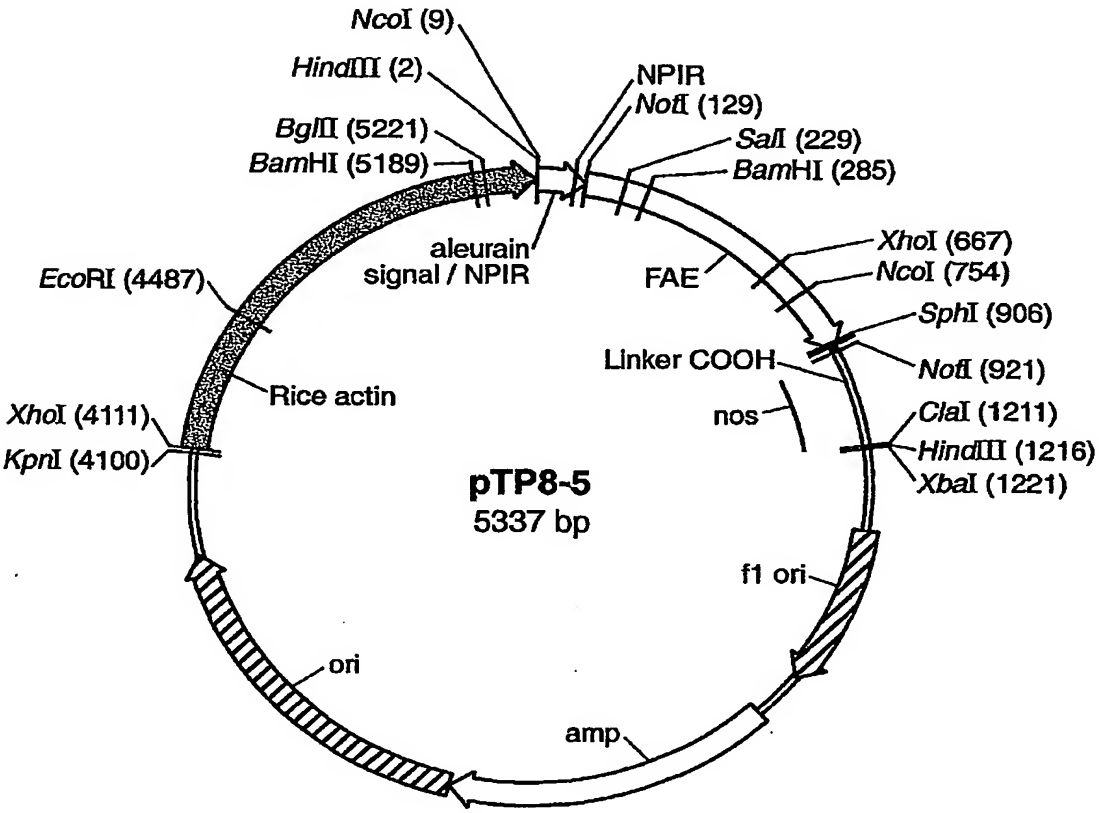

NcoI (9)
HindIII (2)
BglII (5221)
BamHI (5189)
NPIR
NotI (129)
SalI (229)
BamHI (285)
aleurain signal / NPIR
FAE
XhoI (667)
NcoI (754)
SphI (906)
NotI (921)
EcoRI (4487)
Linker COOH
Rice actin
nos
XhoI (4111)
KpnI (4100)
ClaI (1211)
HindIII (1216)
XbaI (1221)
pTP8-5
5337 bp
f1 ori
ori
amp

FIG._36A

```
             NcoI
            ~~~~~~~
     HindIII
     ~~~~~~
                M  A  H  A   R  V  L   L  L  A   L  A  V  L   A  T  A   A  V  A V
   1 AAGCTTACCA TGGCCCACGC CCGCGTCCTC CTCCTGGCGC TCGCCGTGCT GGCCACGGCC GCCGTCGCCG
                                                                   NotI
                                                                 ~~~~~~~~~
     •  A  S  S   S  S  F   A  D  S   N  P  I  R   P  V  T   D  R  A   A  A  S  T •
  71 TCGCCTCCTC CTCCTCCTTC GCCGACTCCA ACCCGATCCG GCCCGTCACC GACCGCGCGG CCGCCTCCAC
     • Q  G  I   S  E  D   L  Y  S  R   L  V  E   M  A  T   I  S  Q  A   A  Y  A
 141 GCAGGGCATC TCCGAAGACC TCTACAGCCG TTTAGTCGAA ATGGCCACTA TCTCCCAAGC TGCCTACGCC
                        SalI
                       ~~~~~~~~
      D  L  C   N  I  P  S   T  I  I   K  G  E   K  I  Y  N   S  Q  T   D  I  N   G
 211 GACCTGTGCA ACATTCCGTC GACTATTATC AAGGGAGAGA AAATTTACAA TTCTCAAACT GACATTAACG
        BamHI
        ~~~~~~~
     •  W  I  L   R  D  D   S  S  K   E  I  I  T   V  F  R   G  T  G   S  D  T  N •
 281 GATGGATCCT CCGCGACGAC AGCAGCAAAG AAATAATCAC CGTCTTCCGT GGCACTGGTA GTGATACGAA
     • L  Q  L   D  T  N   Y  T  L  T   P  F  D   T  L  P   Q  C  N  G   C  E  V
 351 TCTACAACTC GATACTAACT ACACCCTCAC GCCTTTCGAC ACCCTACCAC AATGCAACGG TTGTGAAGTA
      H  G  G   Y  Y  I  G   W  V  S   V  Q  D   Q  V  E  S   L  V  K   Q  Q  V   S
 421 CACGGTGGAT ATTATATTGG ATGGGTCTCC GTCCAGGACC AAGTCGAGTC GCTTGTCAAA CAGCAGGTTA
     •  Q  Y  P   D  Y  A   L  T  V   T  G  H  X   L  G  A   S  L  A   A  L  T  A •
 491 GCCAGTATCC GGACTACGCG CTGACCGTGA CCGGCCACKC CCTCGGCGCC TCCCTGGCGG CACTCACTGC
     • A  Q  L   S  A  T   Y  D  N  I   R  L  Y   T  F  G   E  P  R  S   G  N  Q
 561 CGCCCAGCTG TCTGCGACAT ACGACAACAT CCGCCTGTAC ACCTTCGGCG AACCGCGCAG CGGCAATCAG
                                            XhoI
                                           ~~~~~~~~
      A  F  A   S  Y  M  N   D  A  F   Q  A  S   S  P  D  T   T  Q  Y   F  R  V   T
 631 GCCTTCGCGT CGTACATGAA CGATGCCTTC CAAGCCTCGA GCCCAGATAC GACGCAGTAT TTCCGGGTCA
                                                               NcoI
                                                              ~~~~~~~
     •  H  A  N   D  G  I   P  N  L   P  P  V  E   Q  G  Y   A  H  G   G  V  E  Y •
```

FIG._36B

```
 701 CTCATGCCAA CGACGGCATC CCAAACCTGC CCCCGGTGGA GCAGGGGTAC GCCCATGGCG GTGTAGAGTA
     · W  S  V   D  P  Y   S  A  Q  N   T  F  V   C  T  G   D  E  V  Q   C  C  E
 771 CTGGAGCGTT GATCCTTACA GCGCCCAGAA CACATTTGTC TGCACTGGGG ATGAAGTGCA GTGCTGTGAG
                                                                        SphI
                                                                       ~~~~
      A  Q  G   G  Q  G  V   N  N  A   H  T  T   Y  F  G  M   T  S  G   A  C  T   W
 841 GCCCAGGGCG GACAGGGTGT GAATAATGCG CACACGACTT ATTTTGGGAT GACGAGCGGC GCATGCACCT
                NotI
               ~~~~~~~~~~
     ·  P  V  A   A  A  *
 911 GGCCGGTCGC GGCCGCGTAA CCACTGAAGG ATGAGCTGTA AAGAAGCAGA TCGTTCAAAC ATTTGGCAAT
 981 AAAGTTTCTT AAGATTGAAT CCTGTTGCCG GTCTTGCGAT GATTATCATA TAATTTCTGT TGAATTACGT
1051 TAAGCATGTA ATAATTAACA TGTAATGCAT GACGTTATTT ATGAGATGGG TTTTTATGAT TAGAGTCCCG
1121 CAATTATACA TTTAATACGC GATAGAAAAC AAAATATAGC GCGCAAACTA GGATAAATTA TCGCGCGCGG
                               HindIII
                               ~~~~~~~~
                         ClaI        XbaI
                        ~~~~~~~~    ~~~~~~~
1191 TGTCATCTAT GTTACTAGAT CGATAAGCTT CTAGAGCGGC CGGTGGAGCT CCAATTCGCC CTATAGTGAG
1261 TCGTATTACG CGCGCTCACT GGCCGTCGTT TTACAACGTC GTGACTGGGA AAACCCTGGC GTTACCCAAC
1331 TTAATCGCCT TGCAGCACAT CCCCCTTTCG CCAGCTGGCG TAATAGCGAA GAGGCCCGCA CCGATCGCCC
1401 TTCCCAACAG TTGCGCAGCC TGAATGGCGA ATGGGACGCG CCCTGTAGCG GCGCATTAAG CGCGGCGGGT
1471 GTGGTGGTTA CGCGCAGCGT GACCGCTACA CTTGCCAGCG CCCTAGCGCC CGCTCCTTTC GCTTTCTTCC
1541 CTTCCTTTCT CGCCACGTTC GCCGGCTTTC CCCGTCAAGC TCTAAATCGG GGGCTCCCTT TAGGGTTCCG
1611 ATTTAGTGCT TTACGGCACC TCGACCCCAA AAAACTTGAT TAGGGTGATG GTTCACGTAG TGGGCCATCG
1681 CCCTGATAGA CGGTTTTTCG CCCTTTGACG TTGGAGTCCA CGTTCTTTAA TAGTGGACTC TTGTTCCAAA
1751 CTGGAACAAC ACTCAACCCT ATCTCGGTCT ATTCTTTTGA TTTATAAGGG ATTTTGCCGA TTTCGGCCTA
1821 TTGGTTAAAA AATGAGCTGA TTTAACAAAA ATTTAACGCG AATTTTAACA AAATATTAAC GCTTACAATT
1891 TAGGTGGCAC TTTTCGGGGA AATGTGCGCG GAACCCCTAT TTGTTTATTT TTCTAAATAC ATTCAAATAT
1961 GTATCCGCTC ATGAGACAAT AACCCTGATA AATGCTTCAA TAATATTGAA AAAGGAAGAG TATGAGTATT
2031 CAACATTTCC GTGTCGCCCT TATTCCCTTT TTTGCGGCAT TTTGCCTTCC TGTTTTTGCT CACCCAGAAA
2101 CGCTGGTGAA AGTAAAAGAT GCTGAAGATC AGTTGGGTGC ACGAGTGGGT TACATCGAAC TGGATCTCAA
2171 CAGCGGTAAG ATCCTTGAGA GTTTTCGCCC CGAAGAACGT TTTCCAATGA TGAGCACTTT TAAAGTTCTG
2241 CTATGTGGCG CGGTATTATC CCGTATTGAC GCCGGGCAAG AGCAACTCGG TCGCCGCATA CACTATTCTC
2311 AGAATGACTT GGTTGAGTAC TCACCAGTCA CAGAAAAGCA TCTTACGGAT GGCATGACAG TAAGAGAATT
```

FIG._36C

```
2381  ATGCAGTGCT GCCATAACCA TGAGTGATAA CACTGCGGCC AACTTACTTC TGACAACGAT CGGAGGACCG
2451  AAGGAGCTAA CCGCTTTTTT GCACAACATG GGGGATCATG TAACTCGCCT TGATCGTTGG GAACCGGAGC
2521  TGAATGAAGC CATACCAAAC GACGAGCGTG ACACCACGAT GCCTGTAGCA ATGGCAACAA CGTTGCGCAA
2591  ACTATTAACT GGCGAACTAC TTACTCTAGC TTCCCGGCAA CAATTAATAG ACTGGATGGA GGCGGATAAA
2661  GTTGCAGGAC CACTTCTGCG CTCGGCCCTT CCGGCTGGCT GGTTTATTGC TGATAAATCT GGAGCCGGTG
2731  AGCGTGGGTC TCGCGGTATC ATTGCAGCAC TGGGGCCAGA TGGTAAGCCC TCCCGTATCG TAGTTATCTA
2801  CACGACGGGG AGTCAGGCAA CTATGGATGA ACGAAATAGA CAGATCGCTG AGATAGGTGC CTCACTGATT
2871  AAGCATTGGT AACTGTCAGA CCAAGTTTAC TCATATATAC TTTAGATTGA TTTAAAACTT CATTTTTAAT
2941  TTAAAAGGAT CTAGGTGAAG ATCCTTTTTG ATAATCTCAT GACCAAAATC CCTTAACGTG AGTTTTCGTT
3011  CCACTGAGCG TCAGACCCCG TAGAAAAGAT CAAAGGATCT TCTTGAGATC CTTTTTTTCT GCGCGTAATC
3081  TGCTGCTTGC AAACAAAAAA ACCACCGCTA CCAGCGGTGG TTTGTTTGCC GGATCAAGAG CTACCAACTC
3151  TTTTTCCGAA GGTAACTGGC TTCAGCAGAG CGCAGATACC AAATACTGTC CTTCTAGTGT AGCCGTAGTT
3221  AGGCCACCAC TTCAAGAACT CTGTAGCACC GCCTACATAC CTCGCTCTGC TAATCCTGTT ACCAGTGGCT
3291  GCTGCCAGTG GCGATAAGTC GTGTCTTACC GGGTTGGACT CAAGACGATA GTTACCGGAT AAGGCGCAGC
3361  GGTCGGGCTG AACGGGGGGT TCGTGCACAC AGCCCAGCTT GGAGCGAACG ACCTACACCG AACTGAGATA
3431  CCTACAGCGT GAGCTATGAG AAAGCGCCAC GCTTCCCGAA GGGAGAAAGG CGGACAGGTA TCCGGTAAGC
3501  GGCAGGGTCG GAACAGGAGA GCGCACGAGG GAGCTTCCAG GGGGAAACGC CTGGTATCTT TATAGTCCTG
3571  TCGGGTTTCG CCACCTCTGA CTTGAGCGTC GATTTTTGTG ATGCTCGTCA GGGGGGCGGA GCCTATGGAA
3641  AAACGCCAGC AACGCGGCCT TTTTACGGTT CCTGGCCTTT TGCTGGCCTT TTGCTCACAT GTTCTTTCCT
3711  GCGTTATCCC CTGATTCTGT GGATAACCGT ATTACCGCCT TTGAGTGAGC TGATACCGCT CGCCGCAGCC
3781  GAACGACCGA GCGCAGCGAG TCAGTGAGCG AGGAAGCGGA AGAGCGCCCA ATACGCAAAC CGCCTCTCCC
3851  CGCGCGTTGG CCGATTCATT AATGCAGCTG GCACGACAGG TTTCCCGACT GGAAAGCGGG CAGTGAGCGC
3921  AACGCAATTA ATGTGAGTTA GCTCACTCAT TAGGCACCCC AGGCTTTACA CTTTATGCTT CCGGCTCGTA
3991  TGTTGTGTGG AATTGTGAGC GGATAACAAT TTCACACAGG AAACAGCTAT GACCATGATT ACGCCAAGCG
                                            KpnI            XhoI
                                           ~~~~~~~~       ~~~~~~
4061  CGCAATTAAC CCTCACTAAA GGGAACAAAA GCTGGGTACC GGGCCCCCCC TCGAGGTCAT TCATATGCTT
4131  GAGAAGAGAG TCGGGATAGT CCAAAATAAA ACAAAGGTAA GATTACCTGG TCAAAAGTGA AAACATCAGT
4201  TAAAAGGTGG TATAAGTAAA ATATCGGTAA TAAAAGGTGG CCCAAAGTGA AATTTACTCT TTTCTACTAT
4271  TATAAAAATT GAGGATGTTT TGTCGGTACT TTGATACGTC ATTTTTGTAT GAATTGGTTT TTAAGTTTAT
4341  TCGCGATTTG GAAATGCATA TCTGTATTTG AGTCGGTTTT TAAGTTCGTT GCTTTTGTAA ATACAGAGGG
4411  ATTTGTATAA GAAATATCTT TAAAAAACCC ATATGCTAAT TTGACATAAT TTTTGAGAAA AATATATATT
          EcoRI
         ~~~~~~~~
4481  CAGGCGAATT CCACAATGAA CAATAATAAG ATTAAAATAG CTTGCCCCCG TTGCAGCGAT GGGTATTTTT
```

FIG._36D

```
4551  TCTAGTAAAA TAAAAGATAA ACTTAGACTC AAAACATTTA CAAAAACAAC CCCTAAAGTC CTAAAGCCCA
4621  AAGTGCTATG CACGATCCAT AGCAAGCCCA GCCCAACCCA ACCCAACCCA ACCCACCCCA GTGCAGCCAA
4691  CTGGCAAATA GTCTCCACCC CCGGCACTAT CACCGTGAGT TGTCCGCACC ACCGCACGTC TCGCAGCCAA
4761  AAAAAAAAAA AGAAAGAAAA AAAAGAAAAA GAAAAACAGC AGGTGGGTCC GGGTCGTGGG GGCCGGAAAA
4831  GCGAGGAGGA TCGCGAGCAG CGACGAGGCC CGGCCCTCCC TCCGCTTCCA AAGAAACGCC CCCCATCGCC
4901  ACTATATACA TACCCCCCCC TCTCCTCCCA TCCCCCCAAC CCTACCACCA CCACCACCAC CACCTCCTCC
4971  CCCCTCGCTG CCGGACGACG AGCTCCTCCC CCCTCCCCCT CCGCCGCCGC CGGTAACCAC CCCGCCCCTC
5041  TCCTCTTTCT TTCTCCGTTT TTTTTTTCGT CTCGGTCTCG ATCTTTGGCC TTGGTAGTTT GGGTGGGCGA
5111  GAGCGGCTTC GTCGCCCAGA TCGGTGCGCG GGAGGGGCGG GATCTCGCGG CTGGCGTCTC CGGGCGTGAG
              BamHI                              BglII
             ~~~~~~~                            ~~~~~~
5181  TCGGCCCGGA TCCTCGCGGG GAATGGGGCT CTCGGATGTA GATCTTCTTT CTTTCTTCTT TTTGTGGTAG
5251  AATTTGAATC CCTCAGCATT GTTCATCGGT AGTTTTTCTT TTCATGATTT GTGACAAATG CAGCCTCGTG
5321  CGGAGCTTTT TTGTAGC
```

FIG._36E

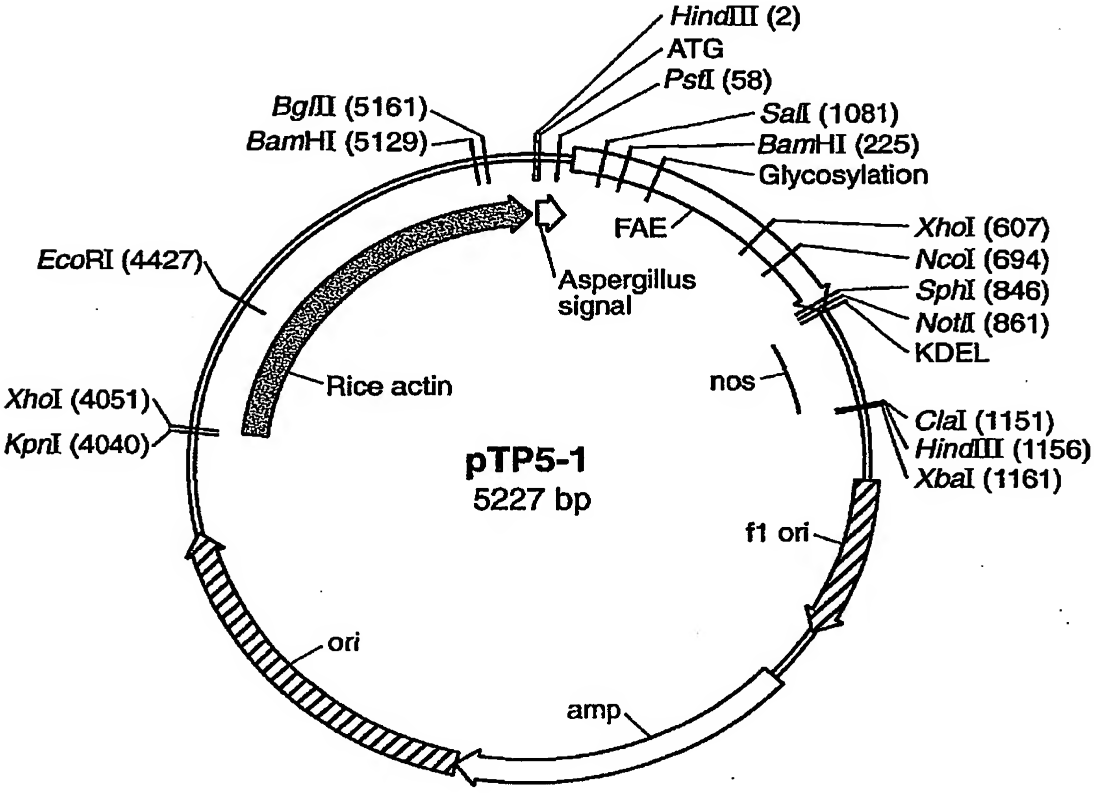

HindIII (2)
ATG
PstI (58)
SalI (1081)
BamHI (225)
Glycosylation
BglII (5161)
BamHI (5129)
FAE
XhoI (607)
NcoI (694)
SphI (846)
NotI (861)
KDEL
EcoRI (4427)
Aspergillus signal
Rice actin
nos
XhoI (4051)
KpnI (4040)
ClaI (1151)
HindIII (1156)
XbaI (1161)
pTP5-1
5227 bp
f1 ori
ori
amp

FIG._37A

```
    HindIII                                                  PstI
    ~~~~~~                                                  ~~~~~~~~
               M  K  Q  F   S  A  K   H  V  L   A  V  V  V   T  A  G   H  A  L   A
  1 AAGCTTAACA TGAAGCAGTT CTCCGCCAAA CACGTCCTCG CAGTTGTGGT GACTGCAGGG CACGCCTTAG
    ·  A  S  T   Q  G  I   S  E  D   L  Y  S  R   L  V  E   M  A  T   I  S  Q  A   ·
 71 CAGCCTCTAC GCAAGGCATC TCCGAAGACC TCTACAGCCG TTTAGTCGAA ATGGCCACTA TCTCCCAAGC
                                 SalI
                                ~~~~~~~~
    · A  Y  A   D  L  C   N  I  P  S   T  I  I   K  G  E   K  I  Y  N   S  Q  T
141 TGCCTACGCC GACCTGTGCA ACATTCCGTC GACTATTATC AAGGGAGAGA AAATTTACAA TTCTCAAACT
                  BamHI
                 ~~~~~~~~
     D  I  N   G  W  I  L   R  D  D   S  S  K   E  I  I  T   V  F  R   G  T  G   S
211 GACATTAACG GATGGATCCT CCGCGACGAC AGCAGCAAAG AAATAATCAC CGTCTTCCGT GGCACTGGTA
    ·  D  T  N   L  Q  L   D  T  N   Y  T  L  T   P  F  D   T  L  P   Q  C  N  G   ·
281 GTGATACGAA TCTACAACTC GATACTAACT ACACCCTCAC GCCTTTCGAC ACCCTACCAC AATGCAACGG
    · C  E  V   H  G  G   Y  Y  I  G   W  V  S   V  Q  D   Q  V  E  S   L  V  K
351 TTGTGAAGTA CACGGTGGAT ATTATATTGG ATGGGTCTCC GTCCAGGACC AAGTCGAGTC GCTTGTCAAA
     Q  Q  V   S  Q  Y  P   D  Y  A   L  T  V   T  G  H  X   L  G  A   S  L  A   A
421 CAGCAGGTTA GCCAGTATCC GGACTACGCG CTGACCGTGA CCGGCCACKC CCTCGGCGCC TCCCTGGCGG
    ·  L  T  A   A  Q  L   S  A  T   Y  D  N  I   R  L  Y   T  F  G   E  P  R  S   ·
491 CACTCACTGC CGCCCAGCTG TCTGCGACAT ACGACAACAT CCGCCTGTAC ACCTTCGGCG AACCGCGCAG
                                                    XhoI
                                                   ~~~~~~~~
    · G  N  Q   A  F  A   S  Y  M  N   D  A  F   Q  A  S   S  P  D  T   T  Q  Y
561 CGGCAATCAG GCCTTCGCGT CGTACATGAA CGATGCCTTC CAAGCCTCGA GCCCAGATAC GACGCAGTAT
                                                                        NcoI
                                                                        ~~
     F  R  V   T  H  A  N   D  G  I   P  N  L   P  P  V  E   Q  G  Y   A  H  G   G
631 TTCCGGGTCA CTCATGCCAA CGACGGCATC CCAAACCTGC CCCCGGTGGA GCAGGGGTAC GCCCATGGCG
    ·  V  E  Y   W  S  V   D  P  Y   S  A  Q  N   T  F  V   C  T  G   D  E  V  Q   ·
701 GTGTAGAGTA CTGGAGCGTT GATCCTTACA GCGCCCAGAA CACATTTGTC TGCACTGGGG ATGAAGTGCA
    · C  C  E   A  Q  G   G  Q  G  V   N  N  A   H  T  T   Y  F  G  M   T  S  G
771 GTGCTGTGAG GCCCAGGGCG GACAGGGTGT GAATAATGCG CACACGACTT ATTTTGGGAT GACGAGCGGC
```

FIG._37B

```
        SphI             NotI
        ~~~~~~          ~~~~~~~~~
       A  C  T   W  P  V  A   A  A  E   P  L  K   D  E  L   *
 841  GCATGCACCT GGCCGGTCGC GGCCGCGGAA CCACTGAAGG ATGAGCTGTA AAGAAGCAGA TCGTTCAAAC
 911  ATTTGGCAAT AAAGTTTCTT AAGATTGAAT CCTGTTGCCG GTCTTGCGAT GATTATCATA TAATTTCTGT
 981  TGAATTACGT TAAGCATGTA ATAATTAACA TGTAATGCAT GACGTTATTT ATGAGATGGG TTTTTATGAT
1051  TAGAGTCCCG CAATTATACA TTTAATACGC GATAGAAAAC AAAATATAGC GCGCAAACTA GGATAAATTA
                                          HindIII
                                          ~~~~~~~
                                    ClaI        XbaI
                                   ~~~~~~      ~~~~~~
1121  TCGCGCGCGG TGTCATCTAT GTTACTAGAT CGATAAGCTT CTAGAGCGGC CGGTGGAGCT CCAATTCGCC
1191  CTATAGTGAG TCGTATTACG CGCGCTCACT GGCCGTCGTT TTACAACGTC GTGACTGGGA AAACCCTGGC
1261  GTTACCCAAC TTAATCGCCT TGCAGCACAT CCCCCTTTCG CCAGCTGGCG TAATAGCGAA GAGGCCCGCA
1331  CCGATCGCCC TTCCCAACAG TTGCGCAGCC TGAATGGCGA ATGGGACGCG CCCTGTAGCG GCGCATTAAG
1401  CGCGGCGGGT GTGGTGGTTA CGCGCAGCGT GACCGCTACA CTTGCCAGCG CCCTAGCGCC CGCTCCTTTC
1471  GCTTTCTTCC CTTCCTTTCT CGCCACGTTC GCCGGCTTTC CCCGTCAAGC TCTAAATCGG GGGCTCCCTT
1541  TAGGGTTCCG ATTTAGTGCT TTACGGCACC TCGACCCCAA AAAACTTGAT TAGGGTGATG GTTCACGTAG
1611  TGGGCCATCG CCCTGATAGA CGGTTTTTCG CCCTTTGACG TTGGAGTCCA CGTTCTTTAA TAGTGGACTC
1681  TTGTTCCAAA CTGGAACAAC ACTCAACCCT ATCTCGGTCT ATTCTTTTGA TTTATAAGGG ATTTTGCCGA
1751  TTTCGGCCTA TTGGTTAAAA AATGAGCTGA TTTAACAAAA ATTTAACGCG AATTTTAACA AAATATTAAC
1821  GCTTACAATT TAGGTGGCAC TTTTCGGGGA AATGTGCGCG GAACCCCTAT TTGTTTATTT TTCTAAATAC
1891  ATTCAAATAT GTATCCGCTC ATGAGACAAT AACCCTGATA AATGCTTCAA TAATATTGAA AAAGGAAGAG
1961  TATGAGTATT CAACATTTCC GTGTCGCCCT TATTCCCTTT TTTGCGGCAT TTTGCCTTCC TGTTTTTGCT
2031  CACCCAGAAA CGCTGGTGAA AGTAAAAGAT GCTGAAGATC AGTTGGGTGC ACGAGTGGGT TACATCGAAC
2101  TGGATCTCAA CAGCGGTAAG ATCCTTGAGA GTTTTCGCCC CGAAGAACGT TTTCCAATGA TGAGCACTTT
2171  TAAAGTTCTG CTATGTGGCG CGGTATTATC CCGTATTGAC GCCGGGCAAG AGCAACTCGG TCGCCGCATA
2241  CACTATTCTC AGAATGACTT GGTTGAGTAC TCACCAGTCA CAGAAAAGCA TCTTACGGAT GGCATGACAG
2311  TAAGAGAATT ATGCAGTGCT GCCATAACCA TGAGTGATAA CACTGCGGCC AACTTACTTC TGACAACGAT
2381  CGGAGGACCG AAGGAGCTAA CCGCTTTTTT GCACAACATG GGGGATCATG TAACTCGCCT TGATCGTTGG
2451  GAACCGGAGC TGAATGAAGC CATACCAAAC GACGAGCGTG ACACCACGAT GCCTGTAGCA ATGGCAACAA
2521  CGTTGCGCAA ACTATTAACT GGCGAACTAC TTACTCTAGC TTCCCGGCAA CAATTAATAG ACTGGATGGA
2591  GGCGGATAAA GTTGCAGGAC CACTTCTGCG CTCGGCCCTT CCGGCTGGCT GGTTTATTGC TGATAAATCT
2661  GGAGCCGGTG AGCGTGGGTC TCGCGGTATC ATTGCAGCAC TGGGGCCAGA TGGTAAGCCC TCCCGTATCG
```

FIG._37C

```
2731  TAGTTATCTA CACGACGGGG AGTCAGGCAA CTATGGATGA ACGAAATAGA CAGATCGCTG AGATAGGTGC
2801  CTCACTGATT AAGCATTGGT AACTGTCAGA CCAAGTTTAC TCATATATAC TTTAGATTGA TTTAAAACTT
2871  CATTTTTAAT TTAAAAGGAT CTAGGTGAAG ATCCTTTTTG ATAATCTCAT GACCAAAATC CCTTAACGTG
2941  AGTTTTCGTT CCACTGAGCG TCAGACCCCG TAGAAAAGAT CAAAGGATCT TCTTGAGATC CTTTTTTTCT
3011  GCGCGTAATC TGCTGCTTGC AAACAAAAAA ACCACCGCTA CCAGCGGTGG TTTGTTTGCC GGATCAAGAG
3081  CTACCAACTC TTTTTCCGAA GGTAACTGGC TTCAGCAGAG CGCAGATACC AAATACTGTC CTTCTAGTGT
3151  AGCCGTAGTT AGGCCACCAC TTCAAGAACT CTGTAGCACC GCCTACATAC CTCGCTCTGC TAATCCTGTT
3221  ACCAGTGGCT GCTGCCAGTG GCGATAAGTC GTGTCTTACC GGGTTGGACT CAAGACGATA GTTACCGGAT
3291  AAGGCGCAGC GGTCGGGCTG AACGGGGGGT TCGTGCACAC AGCCCAGCTT GGAGCGAACG ACCTACACCG
3361  AACTGAGATA CCTACAGCGT GAGCTATGAG AAAGCGCCAC GCTTCCCGAA GGGAGAAAGG CGGACAGGTA
3431  TCCGGTAAGC GGCAGGGTCG GAACAGGAGA GCGCACGAGG GAGCTTCCAG GGGGAAACGC CTGGTATCTT
3501  TATAGTCCTG TCGGGTTTCG CCACCTCTGA CTTGAGCGTC GATTTTTGTG ATGCTCGTCA GGGGGGCGGA
3571  GCCTATGGAA AAACGCCAGC AACGCGGCCT TTTTACGGTT CCTGGCCTTT TGCTGGCCTT TTGCTCACAT
3641  GTTCTTTCCT GCGTTATCCC CTGATTCTGT GGATAACCGT ATTACCGCCT TTGAGTGAGC TGATACCGCT
3711  CGCCGCAGCC GAACGACCGA GCGCAGCGAG TCAGTGAGCG AGGAAGCGGA AGAGCGCCCA ATACGCAAAC
3781  CGCCTCTCCC CGCGCGTTGG CCGATTCATT AATGCAGCTG GCACGACAGG TTTCCCGACT GGAAAGCGGG
3851  CAGTGAGCGC AACGCAATTA ATGTGAGTTA GCTCACTCAT TAGGCACCCC AGGCTTTACA CTTTATGCTT
3921  CCGGCTCGTA TGTTGTGTGG AATTGTGAGC GGATAACAAT TTCACACAGG AAACAGCTAT GACCATGATT
                                                     KpnI              XhoI
                                                    ~~~~~~~~         ~~~~~~~
3991  ACGCCAAGCG CGCAATTAAC CCTCACTAAA GGGAACAAAA GCTGGGTACC GGGCCCCCCC TCGAGGTCAT
4061  TCATATGCTT GAGAAGAGAG TCGGGATAGT CCAAAATAAA ACAAAGGTAA GATTACCTGG TCAAAAGTGA
4131  AAACATCAGT TAAAAGGTGG TATAAGTAAA ATATCGGTAA TAAAAGGTGG CCCAAAGTGA AATTTACTCT
4201  TTTCTACTAT TATAAAAATT GAGGATGTTT TGTCGGTACT TTGATACGTC ATTTTTGTAT GAATTGGTTT
4271  TTAAGTTTAT TCGCGATTTG GAAATGCATA TCTGTATTTG AGTCGGTTTT TAAGTTCGTT GCTTTTGTAA
4341  ATACAGAGGG ATTTGTATAA GAAATATCTT TAAAAAACCC ATATGCTAAT TTGACATAAT TTTTGAGAAA
                      EcoRI
                      ~~~~~~~~
4411  AATATATATT CAGGCGAATT CCACAATGAA CAATAATAAG ATTAAAATAG CTTGCCCCCG TTGCAGCGAT
4481  GGGTATTTTT TCTAGTAAAA TAAAAGATAA ACTTAGACTC AAAACATTTA CAAAAACAAC CCCTAAAGTC
4551  CTAAAGCCCA AAGTGCTATG CACGATCCAT AGCAAGCCCA GCCCAACCCA ACCCAACCCA ACCCACCCCA
4621  GTGCAGCCAA CTGGCAAATA GTCTCCACCC CCGGCACTAT CACCGTGAGT TGTCCGCACC ACCGCACGTC
4691  TCGCAGCCAA AAAAAAAAAA AGAAAGAAAA AAAAGAAAAA GAAAAACAGC AGGTGGGTCC GGGTCGTGGG
```

FIG._37D

```
4761  GGCCGGAAAA GCGAGGAGGA TCGCGAGCAG CGACGAGGCC CGGCCCTCCC TCCGCTTCCA AAGAAACGCC
4831  CCCCATCGCC ACTATATACA TACCCCCCCC TCTCCTCCCA TCCCCCCAAC CCTACCACCA CCACCACCAC
4901  CACCTCCTCC CCCCTCGCTG CCGGACGACG AGCTCCTCCC CCCTCCCCCT CCGCCGCCGC CGGTAACCAC
4971  CCCGCCCCTC TCCTCTTTCT TTCTCCGTTT TTTTTTTCGT CTCGGTCTCG ATCTTTGGCC TTGGTAGTTT
5041  GGGTGGGCGA GAGCGGCTTC GTCGCCCAGA TCGGTGCGCG GGAGGGGCGG GATCTCGCGG CTGGCGTCTC
                       BamHI                           BglII
                       ~~~~~~~~                        ~~~~~~~
5111  CGGGCGTGAG TCGGCCCGGA TCCTCGCGGG GAATGGGGCT CTCGGATGTA GATCTTCTTT CTTTCTTCTT
5181  TTTGTGGTAG AATTTGAATC CCTCAGCATT GTTCATCGGT AGTTTTTCTT TTCATGATTT GTGACAAATG
5251  CAGCCTCGTG CGGAGCTTTT TTGTAGC
```

FIG._37E

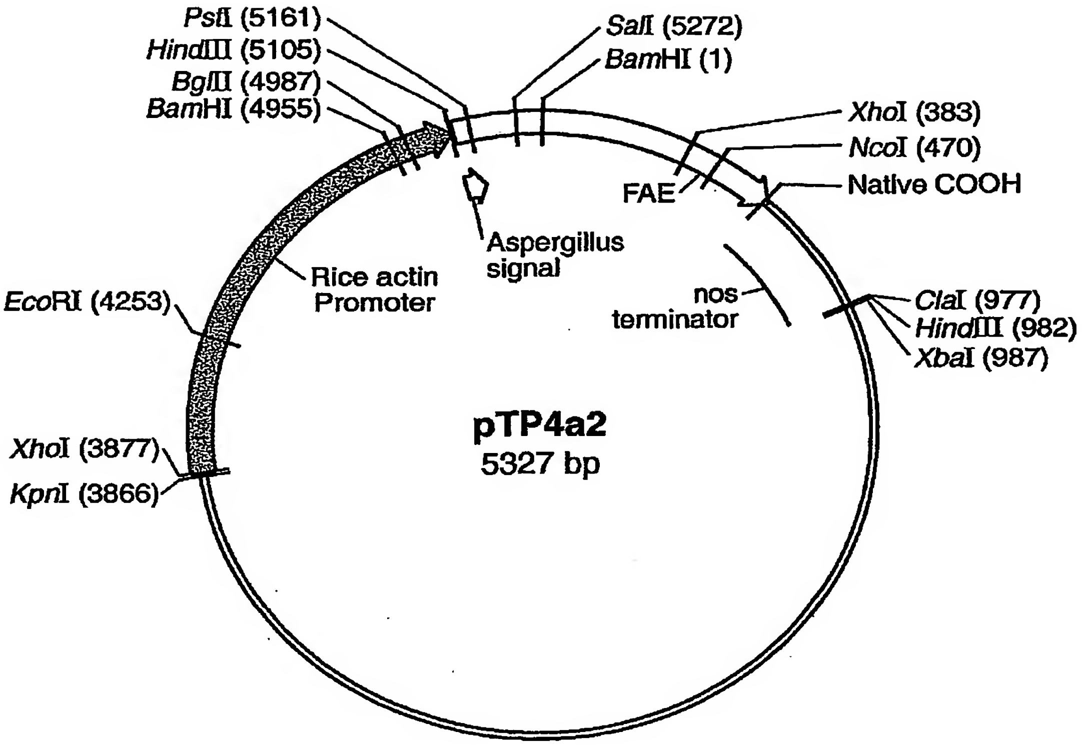

PstI (5161)
HindIII (5105)
BglII (4987)
BamHI (4955)
SalI (5272)
BamHI (1)
XhoI (383)
NcoI (470)
FAE
Native COOH
Aspergillus signal
Rice actin Promoter
EcoRI (4253)
nos terminator
ClaI (977)
HindIII (982)
XbaI (987)
pTP4a2
5327 bp
XhoI (3877)
KpnI (3866)

FIG._38A

```
                                      BamHI
                                      ~~~~~~
     · I  L  R   D  D  S   S  K  E  I   I  T  V   F  R  G   T  G  S  D   T  N  L
   1 GATCCTCCGC GACGACAGCA GCAAAGAAAT AATCACCGTC TTCCGTGGCA CTGGTAGTGA TACGAATCTA
      Q  L  D   T  N  Y  T   L  T  P   F  D  T   L  P  Q  C   N  G  C   E  V  H   G
  71 CAACTCGATA CTAACTACAC CCTCACGCCT TTCGACACCC TACCACAATG CAACGGTTGT GAAGTACACG
     ·  G  Y  Y   I  G  W   V  S  V   Q  D  Q  V   E  S  L   V  K  Q   Q  V  S  Q ·
 141 GTGGATATTA TATTGGATGG GTCTCCGTCC AGGACCAAGT CGAGTCGCTT GTCAAACAGC AGGTTAGCCA
     · Y  P  D   Y  A  L   T  V  T  G   H  X  L   G  A  S   L  A  A  L   T  A  A
 211 GTATCCGGAC TACGCGCTGA CCGTGACCGG CCACKCCCTC GGCGCCTCCC TGGCGGCACT CACTGCCGCC
      Q  L  S   A  T  Y  D   N  I  R   L  Y  T   F  G  E  P   R  S  G   N  Q  A   F
 281 CAGCTGTCTG CGACATACGA CAACATCCGC CTGTACACCT TCGGCGAACC GCGCAGCGGC AATCAGGCCT
                                                            XhoI
                                                         ~~~~~~~~
     ·  A  S  Y   M  N  D   A  F  Q   A  S  S  P   D  T  T   Q  Y  F   R  V  T  H ·
 351 TCGCGTCGTA CATGAACGAT GCCTTCCAAG CCTCGAGCCC AGATACGACG CAGTATTTCC GGGTCACTCA
                                                                 NcoI
                                                                ~~~~~~~
     · A  N  D   G  I  P   N  L  P  P   V  E  Q   G  Y  A   H  G  G  V   E  Y  W
 421 TGCCAACGAC GGCATCCCAA ACCTGCCCCC GGTGGAGCAG GGGTACGCCC ATGGCGGTGT AGAGTACTGG
      S  V  D   P  Y  S  A   Q  N  T   F  V  C   T  G  D  E   V  Q  C   C  E  A   Q
 491 AGCGTTGATC CTTACAGCGC CCAGAACACA TTTGTCTGCA CTGGGGATGA AGTGCAGTGC TGTGAGGCCC
     ·  G  G  Q   G  V  N   N  A  H   T  T  Y  F   G  M  T   S  G  A   C  T  W  * ·
 561 AGGGCGGACA GGGTGTGAAT AATGCGCACA CGACTTATTT TGGGATGACG AGCGGAGCCT GTACATGGTG
     · *
 631 ATCAGTCATT TCAGCCTCCC CGAGTGTACC AGGAAAGATG GATGTCCTGG AGAGGGGGCC GCGTAACCAC
 701 TGAAGGATGA GCTGTAAAGA AGCAGATCGT TCAAACATTT GGCAATAAAG TTTCTTAAGA TTGAATCCTG
 771 TTGCCGGTCT TGCGATGATT ATCATATAAT TTCTGTTGAA TTACGTTAAG CATGTAATAA TTAACATGTA
 841 ATGCATGACG TTATTTATGA GATGGGTTTT TATGATTAGA GTCCCGCAAT TATACATTTA ATACGCGATA
                                                                          ClaI
 911 GAAAACAAAA TATAGCGCGC AAACTAGGAT AAATTATCGC GCGCGGTGTC ATCTATGTTA CTAGATCGAT
                                      XbaI
                                    ~~~~~~~~
                                   HindIII
                                   ~~~~~~~
```

FIG._38B

```
 981  AAGCTTCTAG AGCGGCCGGT GGAGCTCCAA TTCGCCCTAT AGTGAGTCGT ATTACGCGCG CTCACTGGCC
1051  GTCGTTTTAC AACGTCGTGA CTGGGAAAAC CCTGGCGTTA CCCAACTTAA TCGCCTTGCA GCACATCCCC
1121  CTTTCGCCAG CTGGCGTAAT AGCGAAGAGG CCCGCACCGA TCGCCCTTCC CAACAGTTGC GCAGCCTGAA
1191  TGGCGAATGG GACGCGCCCT GTAGCGGCGC ATTAAGCGCG GCGGGTGTGG TGGTTACGCG CAGCGTGACC
1261  GCTACACTTG CCAGCGCCCT AGCGCCCGCT CCTTTCGCTT TCTTCCCTTC CTTTCTCGCC ACGTTCGCCG
1331  GCTTTCCCCG TCAAGCTCTA AATCGGGGGC TCCCTTTAGG GTTCCGATTT AGTGCTTTAC GGCACCTCGA
1401  CCCCAAAAAA CTTGATTAGG GTGATGGTTC ACGTAGTGGG CCATCGCCCT GATAGACGGT TTTTCGCCCT
1471  TTGACGTTGG AGTCCACGTT CTTTAATAGT GGACTCTTGT TCCAAACTGG AACAACACTC AACCCTATCT
1541  CGGTCTATTC TTTTGATTTA TAAGGGATTT TGCCGATTTC GGCCTATTGG TTAAAAAATG AGCTGATTTA
1611  ACAAAAATTT AACGCGAATT TTAACAAAAT ATTAACGCTT ACAATTTAGG TGGCACTTTT CGGGGAAATG
1681  TGCGCGGAAC CCCTATTTGT TTATTTTTCT AAATACATTC AAATATGTAT CCGCTCATGA GACAATAACC
1751  CTGATAAATG CTTCAATAAT ATTGAAAAAG GAAGAGTATG AGTATTCAAC ATTTCCGTGT CGCCCTTATT
1821  CCCTTTTTTG CGGCATTTTG CCTTCCTGTT TTTGCTCACC CAGAAACGCT GGTGAAAGTA AAAGATGCTG
1891  AAGATCAGTT GGGTGCACGA GTGGGTTACA TCGAACTGGA TCTCAACAGC GGTAAGATCC TTGAGAGTTT
1961  TCGCCCCGAA GAACGTTTTC CAATGATGAG CACTTTTAAA GTTCTGCTAT GTGGCGCGGT ATTATCCCGT
2031  ATTGACGCCG GGCAAGAGCA ACTCGGTCGC CGCATACACT ATTCTCAGAA TGACTTGGTT GAGTACTCAC
2101  CAGTCACAGA AAAGCATCTT ACGGATGGCA TGACAGTAAG AGAATTATGC AGTGCTGCCA TAACCATGAG
2171  TGATAACACT GCGGCCAACT TACTTCTGAC AACGATCGGA GGACCGAAGG AGCTAACCGC TTTTTTGCAC
2241  AACATGGGGG ATCATGTAAC TCGCCTTGAT CGTTGGGAAC CGGAGCTGAA TGAAGCCATA CCAAACGACG
2311  AGCGTGACAC CACGATGCCT GTAGCAATGG CAACAACGTT GCGCAAACTA TTAACTGGCG AACTACTTAC
2381  TCTAGCTTCC CGGCAACAAT TAATAGACTG GATGGAGGCG GATAAAGTTG CAGGACCACT TCTGCGCTCG
2451  GCCCTTCCGG CTGGCTGGTT TATTGCTGAT AAATCTGGAG CCGGTGAGCG TGGGTCTCGC GGTATCATTG
2521  CAGCACTGGG GCCAGATGGT AAGCCCTCCC GTATCGTAGT TATCTACACG ACGGGGAGTC AGGCAACTAT
2591  GGATGAACGA AATAGACAGA TCGCTGAGAT AGGTGCCTCA CTGATTAAGC ATTGGTAACT GTCAGACCAA
2661  GTTTACTCAT ATATACTTTA GATTGATTTA AAACTTCATT TTTAATTTAA AAGGATCTAG GTGAAGATCC
2731  TTTTTGATAA TCTCATGACC AAAATCCCTT AACGTGAGTT TTCGTTCCAC TGAGCGTCAG ACCCCGTAGA
2801  AAAGATCAAA GGATCTTCTT GAGATCCTTT TTTTCTGCGC GTAATCTGCT GCTTGCAAAC AAAAAAACCA
2871  CCGCTACCAG CGGTGGTTTG TTTGCCGGAT CAAGAGCTAC CAACTCTTTT TCCGAAGGTA ACTGGCTTCA
2941  GCAGAGCGCA GATACCAAAT ACTGTCCTTC TAGTGTAGCC GTAGTTAGGC CACCACTTCA AGAACTCTGT
3011  AGCACCGCCT ACATACCTCG CTCTGCTAAT CCTGTTACCA GTGGCTGCTG CCAGTGGCGA TAAGTCGTGT
3081  CTTACCGGGT TGGACTCAAG ACGATAGTTA CCGGATAAGG CGCAGCGGTC GGGCTGAACG GGGGGTTCGT
3151  GCACACAGCC CAGCTTGGAG CGAACGACCT ACACCGAACT GAGATACCTA CAGCGTGAGC TATGAGAAAG
3221  CGCCACGCTT CCCGAAGGGA GAAAGGCGGA CAGGTATCCG GTAAGCGGCA GGGTCGGAAC AGGAGAGCGC
3291  ACGAGGGAGC TTCCAGGGGG AAACGCCTGG TATCTTTATA GTCCTGTCGG GTTTCGCCAC CTCTGACTTG
3361  AGCGTCGATT TTTGTGATGC TCGTCAGGGG GGCGGAGCCT ATGGAAAAAC GCCAGCAACG CGGCCTTTTT
```

FIG._38C

```
3431  ACGGTTCCTG GCCTTTTGCT GGCCTTTTGC TCACATGTTC TTTCCTGCGT TATCCCCTGA TTCTGTGGAT
3501  AACCGTATTA CCGCCTTTGA GTGAGCTGAT ACCGCTCGCC GCAGCCGAAC GACCGAGCGC AGCGAGTCAG
3571  TGAGCGAGGA AGCGGAAGAG CGCCCAATAC GCAAACCGCC TCTCCCCGCG CGTTGGCCGA TTCATTAATG
3641  CAGCTGGCAC GACAGGTTTC CCGACTGGAA AGCGGGCAGT GAGCGCAACG CAATTAATGT GAGTTAGCTC
3711  ACTCATTAGG CACCCCAGGC TTTACACTTT ATGCTTCCGG CTCGTATGTT GTGTGGAATT GTGAGCGGAT
3781  AACAATTTCA CACAGGAAAC AGCTATGACC ATGATTACGC CAAGCGCGCA ATTAACCCTC ACTAAAGGGA
                                    KpnI             XhoI
                                   ~~~~~~~         ~~~~~~~~
3851  ACAAAAGCTG GGTACCGGGC CCCCCCTCGA GGTCATTCAT ATGCTTGAGA AGAGAGTCGG GATAGTCCAA
3921  AATAAAACAA AGGTAAGATT ACCTGGTCAA AAGTGAAAAC ATCAGTTAAA AGGTGGTATA AGTAAAATAT
3991  CGGTAATAAA AGGTGGCCCA AAGTGAAATT TACTCTTTTC TACTATTATA AAAATTGAGG ATGTTTTGTC
4061  GGTACTTTGA TACGTCATTT TTGTATGAAT TGGTTTTTAA GTTTATTCGC GATTTGGAAA TGCATATCTG
4131  TATTTGAGTC GGTTTTTAAG TTCGTTGCTT TTGTAAATAC AGAGGGATTT GTATAAGAAA TATCTTTAAA
                                                                     EcoRI
                                                                    ~~~~~~~~
4201  AAACCCATAT GCTAATTTGA CATAATTTTT GAGAAAAATA TATATTCAGG CGAATTCCAC AATGAACAAT
4271  AATAAGATTA AAATAGCTTG CCCCCGTTGC AGCGATGGGT ATTTTTTCTA GTAAAATAAA AGATAAACTT
4341  AGACTCAAAA CATTTACAAA AACAACCCCT AAAGTCCTAA AGCCCAAAGT GCTATGCACG ATCCATAGCA
4411  AGCCCAGCCC AACCCAACCC AACCCAACCC ACCCCAGTGC AGCCAACTGG CAAATAGTCT CCACCCCCGG
4481  CACTATCACC GTGAGTTGTC CGCACCACCG CACGTCTCGC AGCCAAAAAA AAAAAAAGAA AGAAAAAAAA
4551  GAAAAAGAAA AACAGCAGGT GGGTCCGGGT CGTGGGGGCC GGAAAAGCGA GGAGGATCGC GAGCAGCGAC
4621  GAGGCCCGGC CCTCCCTCCG CTTCCAAAGA AACGCCCCCC ATCGCCACTA TATACATACC CCCCCCTCTC
4691  CTCCCATCCC CCCAACCCTA CCACCACCAC CACCACCACC TCCTCCCCCC TCGCTGCCGG ACGACGAGCT
4761  CCTCCCCCCT CCCCCTCCGC CGCCGCCGGT AACCACCCCG CCCCTCTCCT CTTTCTTTCT CCGTTTTTTT
4831  TTTCGTCTCG GTCTCGATCT TTGGCCTTGG TAGTTTGGGT GGGCGAGAGC GGCTTCGTCG CCCAGATCGG
                                                                    BamHI
                                                                   ~~~~~~~~
4901  TGCGCGGGAG GGGCGGGATC TCGCGGCTGG CGTCTCCGGG CGTGAGTCGG CCCGGATCCT CGCGGGGAAT
                                                 BglII
                                                ~~~~~~~~
4971  GGGGCTCTCG GATGTAGATC TTCTTTCTTT CTTCTTTTTG TGGTAGAATT TGAATCCCTC AGCATTGTTC
                                                                           HindIII
                                                                               ~
5041  ATCGGTAGTT TTTCTTTTCA TGATTTGTGA CAAATGCAGC CTCGTGCGGA GCTTTTTTGT AGCAAGCTTA
```

FIG._38D

```
                                                                     PstI
                                                                 ~~~~~~~
         M  K  Q   F  S  A   K  H  V   L  A  V  V   V  T  A   G  H  A   L  A  A  S·
5111  ACATGAAGCA GTTCTCCGCC AAACACGTCC TCGCAGTTGT GGTGACTGCA GGGCACGCCT TAGCAGCCTC
       ·T  Q  G   I  S  E   D  L  Y  S   R  L  V   E  M  A   T  I  S  Q   A  A  Y
5181  TACGCAAGGC ATCTCCGAAG ACCTCTACAG CCGTTTAGTC GAAATGGCCA CTATCTCCCA AGCTGCCTAC
                                                     SalI
                                                    ~~~~~~
       A  D  L   C  N  I  P   S  T  I   I  K  G   E  K  I  Y   N  S  Q   T  D  I  N
5251  GCCGACCTGT GCAACATTCC GTCGACTATT ATCAAGGGAG AGAAAATTTA CAATTCTCAA ACTGACATTA
            B
            ~
      ·  G  W
5321  ACGGATG
```

FIG._38E

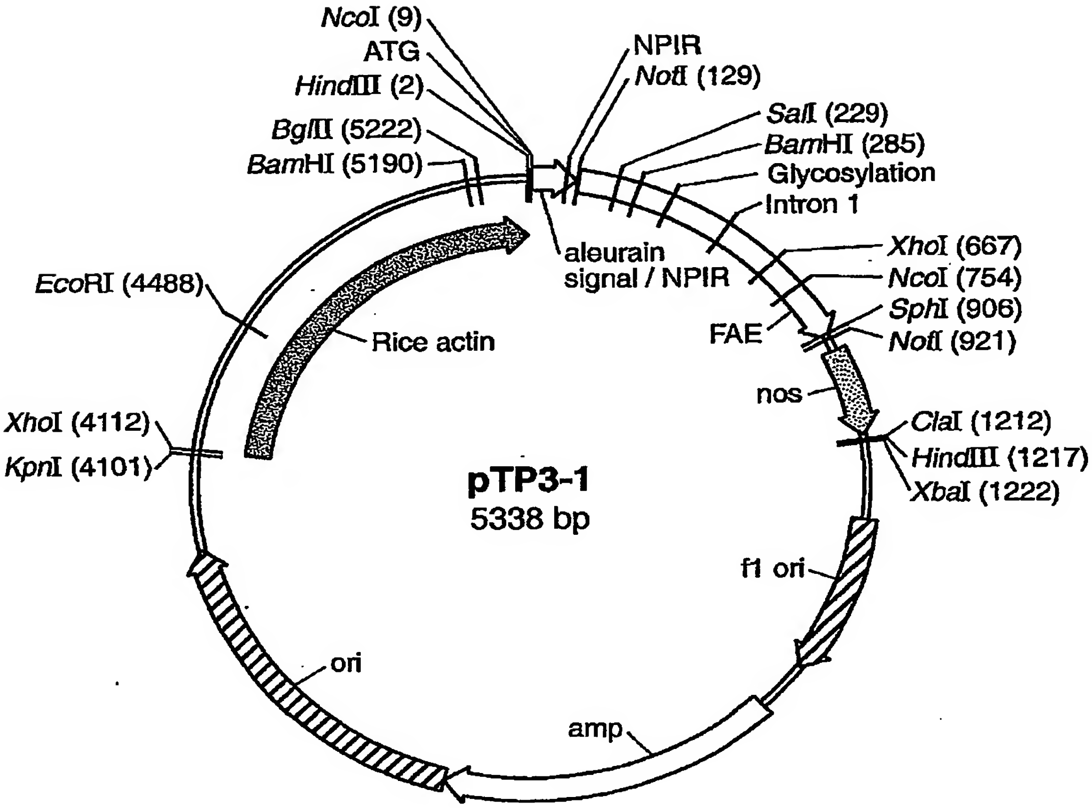

NcoI (9)
ATG
HindIII (2)
BglII (5222)
BamHI (5190)
NPIR
NotI (129)
SalI (229)
BamHI (285)
Glycosylation
Intron 1
aleurain signal / NPIR
XhoI (667)
NcoI (754)
SphI (906)
NotI (921)
FAE
EcoRI (4488)
Rice actin
nos
ClaI (1212)
HindIII (1217)
XbaI (1222)
XhoI (4112)
KpnI (4101)
pTP3-1
5338 bp
f1 ori
ori
amp

FIG._39A

```
             NcoI
           ~~~~~~~
    HindIII
    ~~~~~~
               M  A  H  A   R  V  L   L  L  A   L  A  V  L   A  T  A   A  V  A   V
  1 AAGCTTACCA TGGCCCACGC CCGCGTCCTC CTCCTGGCGC TCGCCGTGCT GGCCACGGCC GCCGTCGCCG

                                         NPIR                      NotI
                                    ~~~~~~~~~~~~~              ~~~~~~~~~
    •  A  S  S   S  S  F   A  D  S   N  P  I  R   P  V  T   D  R  A   A  A  S  T •
 71 TCGCCTCCTC CTCCTCCTTC GCCGACTCCA ACCCGATCCG GCCCGTCACC GACCGCGCGG CCGCCTCCAC
    • Q  G  I   S  E  D   L  Y  S  R   L  V  E   M  A  T   I  S  Q  A   A  Y  A
141 GCAGGGCATC TCCGAAGACC TCTACAGCCG TTTAGTCGAA ATGGCCACTA TCTCCCAAGC TGCCTACGCC

                       SalI
                      ~~~~~~~
     D  L  C   N  I  P  S   T  I  I   K  G  E   K  I  Y  N   S  Q  T   D  I  N   G
211 GACCTGTGCA ACATTCCGTC GACTATTATC AAGGGAGAGA AAATTTACAA TTCTCAAACT GACATTAACG

       BamHI
      ~~~~~~
    •  W  I  L   R  D  D   S  S  K   E  I  I  T   V  F  R   G  T  G   S  D  T  N •
281 GATGGATCCT CCGCGACGAC AGCAGCAAAG AAATAATCAC CGTCTTCCGT GGCACTGGTA GTGATACGAA

                     Glycosylation
                     ~~~~
    • L  Q  L   D  T  N   Y  T  L  T   P  F  D   T  L  P   Q  C  N  G   C  E  V
351 TCTACAACTC GATACTAACT ACACCCTCAC GCCTTTCGAC ACCCTACCAC AATGCAACGG TTGTGAAGTA
     H  G  G   Y  Y  I  G   W  V  S   V  Q  D   Q  V  E  S   L  V  K   Q  Q  V   S
421 CACGGTGGAT ATTATATTGG ATGGGTCTCC GTCCAGGACC AAGTCGAGTC GCTTGTCAAA CAGCAGGTTA
    •  Q  Y  P   D  Y  A   L  T  V   T  G  H  X   L  G  A   S  L  A   A  L  T  A •
491 GCCAGTATCC GGACTACGCG CTGACCGTGA CCGGCCACKC CCTCGGCGCC TCCCTGGCGG CACTCACTGC
    • A  Q  L   S  A  T   Y  D  N  I   R  L  Y   T  F  G   E  P  R  S   G  N  Q
561 CGCCCAGCTG TCTGCGACAT ACGACAACAT CCGCCTGTAC ACCTTCGGCG AACCGCGCAG CGGCAATCAG
```

FIG._39B

```
                                    XhoI
                                  ~~~~~~~
      A  F  A  S   Y  M  N   D  A  F   Q  A  S  S   P  D  T   T  Q  Y   F  R  V  T
 631  GCCTTCGCGT CGTACATGAA CGATGCCTTC CAAGCCTCGA GCCCAGATAC GACGCAGTAT TTCCGGGTCA

                                                             NcoI
                                                           ~~~~~~~
      · H  A  N   D  G  I   P  N  L  P   P  V  E   Q  G  Y   A  H  G  G   V  E  Y ·
 701  CTCATGCCAA CGACGGCATC CCAAACCTGC CCCCGGTGGA GCAGGGGTAC GCCCATGGCG GTGTAGAGTA
      · W  S  V   D  P  Y  S   A  Q  N   T  F  V   C  T  G  D   E  V  Q   C  C  E
 771  CTGGAGCGTT GATCCTTACA GCGCCCAGAA CACATTTGTC TGCACTGGGG ATGAAGTGCA GTGCTGTGAG

                                                                   SphI
                                                                   ~~~~
      A  Q  G  G   Q  G  V   N  N  A   H  T  T  Y   F  G  M   T  S  G   A  C  T  W
 841  GCCCAGGGCG GACAGGGTGT GAATAATGCG CACACGACTT ATTTTGGGAT GACGAGCGGC GCATGCACCT

                 NotI                KDEL
              ~~~~~~~~~~        ~~~~~~~~~~~~~~
      · P  V  A   A  A  E   T  T  E  G  *
 911  GGCCGGTCGC GGCCGCGGAA ACCACTGAAG GATGAGCTGT AAAGAAGCAG ATCGTTCAAA CATTTGGCAA
 981  TAAAGTTTCT TAAGATTGAA TCCTGTTGCC GGTCTTGCGA TGATTATCAT ATAATTTCTG TTGAATTACG
1051  TTAAGCATGT AATAATTAAC ATGTAATGCA TGACGTTATT TATGAGATGG GTTTTTATGA TTAGAGTCCC
1121  GCAATTATAC ATTTAATACG CGATAGAAAA CAAAATATAG CGCGCAAACT AGGATAAATT ATCGCGCGCG

                            HindIII
                            ~~~~~~~
                        ClaI        XbaI
                       ~~~~~~      ~~~~~~
1191  GTGTCATCTA TGTTACTAGA TCGATAAGCT TCTAGAGCGG CCGGTGGAGC TCCAATTCGC CCTATAGTGA
1261  GTCGTATTAC GCGCGCTCAC TGGCCGTCGT TTTACAACGT CGTGACTGGG AAAACCCTGG CGTTACCCAA
1331  CTTAATCGCC TTGCAGCACA TCCCCCTTTC GCCAGCTGGC GTAATAGCGA AGAGGCCCGC ACCGATCGCC
1401  CTTCCCAACA GTTGCGCAGC CTGAATGGCG AATGGGACGC GCCCTGTAGC GGCGCATTAA GCGCGGCGGG
```

FIG._39C

```
1471  TGTGGTGGTT ACGCGCAGCG TGACCGCTAC ACTTGCCAGC GCCCTAGCGC CCGCTCCTTT CGCTTTCTTC
1541  CCTTCCTTTC TCGCCACGTT CGCCGGCTTT CCCCGTCAAG CTCTAAATCG GGGGCTCCCT TTAGGGTTCC
1611  GATTTAGTGC TTTACGGCAC CTCGACCCCA AAAAACTTGA TTAGGGTGAT GGTTCACGTA GTGGGCCATC
1681  GCCCTGATAG ACGGTTTTTC GCCCTTTGAC GTTGGAGTCC ACGTTCTTTA ATAGTGGACT CTTGTTCCAA
1751  ACTGGAACAA CACTCAACCC TATCTCGGTC TATTCTTTTG ATTTATAAGG GATTTTGCCG ATTTCGGCCT
1821  ATTGGTTAAA AAATGAGCTG ATTTAACAAA AATTTAACGC GAATTTTAAC AAAATATTAA CGCTTACAAT
1891  TTAGGTGGCA CTTTTCGGGG AAATGTGCGC GGAACCCCTA TTTGTTTATT TTTCTAAATA CATTCAAATA
1961  TGTATCCGCT CATGAGACAA TAACCCTGAT AAATGCTTCA ATAATATTGA AAAAGGAAGA GTATGAGTAT
2031  TCAACATTTC CGTGTCGCCC TTATTCCCTT TTTTGCGGCA TTTTGCCTTC CTGTTTTTGC TCACCCAGAA
2101  ACGCTGGTGA AAGTAAAAGA TGCTGAAGAT CAGTTGGGTG CACGAGTGGG TTACATCGAA CTGGATCTCA
2171  ACAGCGGTAA GATCCTTGAG AGTTTTCGCC CCGAAGAACG TTTTCCAATG ATGAGCACTT TTAAAGTTCT
2241  GCTATGTGGC GCGGTATTAT CCCGTATTGA CGCCGGGCAA GAGCAACTCG GTCGCCGCAT ACACTATTCT
2311  CAGAATGACT TGGTTGAGTA CTCACCAGTC ACAGAAAAGC ATCTTACGGA TGGCATGACA GTAAGAGAAT
2381  TATGCAGTGC TGCCATAACC ATGAGTGATA ACACTGCGGC CAACTTACTT CTGACAACGA TCGGAGGACC
2451  GAAGGAGCTA ACCGCTTTTT TGCACAACAT GGGGGATCAT GTAACTCGCC TTGATCGTTG GGAACCGGAG
2521  CTGAATGAAG CCATACCAAA CGACGAGCGT GACACCACGA TGCCTGTAGC AATGGCAACA ACGTTGCGCA
2591  AACTATTAAC TGGCGAACTA CTTACTCTAG CTTCCCGGCA ACAATTAATA GACTGGATGG AGGCGGATAA
2661  AGTTGCAGGA CCACTTCTGC GCTCGGCCCT TCCGGCTGGC TGGTTTATTG CTGATAAATC TGGAGCCGGT
2731  GAGCGTGGGT CTCGCGGTAT CATTGCAGCA CTGGGGCCAG ATGGTAAGCC CTCCCGTATC GTAGTTATCT
2801  ACACGACGGG GAGTCAGGCA ACTATGGATG AACGAAATAG ACAGATCGCT GAGATAGGTG CCTCACTGAT
2871  TAAGCATTGG TAACTGTCAG ACCAAGTTTA CTCATATATA CTTTAGATTG ATTTAAAACT TCATTTTTAA
2941  TTTAAAAGGA TCTAGGTGAA GATCCTTTTT GATAATCTCA TGACCAAAAT CCCTTAACGT GAGTTTTCGT
3011  TCCACTGAGC GTCAGACCCC GTAGAAAAGA TCAAAGGATC TTCTTGAGAT CCTTTTTTTC TGCGCGTAAT
3081  CTGCTGCTTG CAAACAAAAA AACCACCGCT ACCAGCGGTG GTTTGTTTGC CGGATCAAGA GCTACCAACT
3151  CTTTTTCCGA AGGTAACTGG CTTCAGCAGA GCGCAGATAC CAAATACTGT CCTTCTAGTG TAGCCGTAGT
3221  TAGGCCACCA CTTCAAGAAC TCTGTAGCAC CGCCTACATA CCTCGCTCTG CTAATCCTGT TACCAGTGGC
3291  TGCTGCCAGT GGCGATAAGT CGTGTCTTAC CGGGTTGGAC TCAAGACGAT AGTTACCGGA TAAGGCGCAG
3361  CGGTCGGGCT GAACGGGGGG TTCGTGCACA CAGCCCAGCT TGGAGCGAAC GACCTACACC GAACTGAGAT
3431  ACCTACAGCG TGAGCTATGA GAAAGCGCCA CGCTTCCCGA AGGGAGAAAG GCGGACAGGT ATCCGGTAAG
3501  CGGCAGGGTC GGAACAGGAG AGCGCACGAG GGAGCTTCCA GGGGGAAACG CCTGGTATCT TTATAGTCCT
3571  GTCGGGTTTC GCCACCTCTG ACTTGAGCGT CGATTTTTGT GATGCTCGTC AGGGGGGCGG AGCCTATGGA
3641  AAAACGCCAG CAACGCGGCC TTTTTACGGT TCCTGGCCTT TTGCTGGCCT TTTGCTCACA TGTTCTTTCC
3711  TGCGTTATCC CCTGATTCTG TGGATAACCG TATTACCGCC TTTGAGTGAG CTGATACCGC TCGCCGCAGC
3781  CGAACGACCG AGCGCAGCGA GTCAGTGAGC GAGGAAGCGG AAGAGCGCCC AATACGCAAA CCGCCTCTCC
3851  CCGCGCGTTG GCCGATTCAT TAATGCAGCT GGCACGACAG GTTTCCCGAC TGGAAAGCGG GCAGTGAGCG
```

FIG._39D

```
3921  CAACGCAATT AATGTGAGTT AGCTCACTCA TTAGGCACCC CAGGCTTTAC ACTTTATGCT TCCGGCTCGT
3991  ATGTTGTGTG GAATTGTGAG CGGATAACAA TTTCACACAG GAAACAGCTA TGACCATGAT TACGCCAAGC
                                            KpnI             XhoI
                                           ~~~~~~~~         ~~~~~~~~
4061  GCGCAATTAA CCCTCACTAA AGGGAACAAA AGCTGGGTAC CGGGCCCCCC CTCGAGGTCA TTCATATGCT
4131  TGAGAAGAGA GTCGGGATAG TCCAAAATAA AACAAAGGTA AGATTACCTG GTCAAAAGTG AAAACATCAG
4201  TTAAAAGGTG GTATAAGTAA AATATCGGTA ATAAAAGGTG GCCCAAAGTG AAATTTACTC TTTTCTACTA
4271  TTATAAAAAT TGAGGATGTT TTGTCGGTAC TTTGATACGT CATTTTTGTA TGAATTGGTT TTTAAGTTTA
4341  TTCGCGATTT GGAAATGCAT ATCTGTATTT GAGTCGGTTT TTAAGTTCGT TGCTTTTGTA AATACAGAGG
4411  GATTTGTATA AGAAATATCT TTAAAAAACC CATATGCTAA TTTGACATAA TTTTTGAGAA AAATATATAT
            EcoRI
            ~~~~~~~~
4481  TCAGGCGAAT TCCACAATGA ACAATAATAA GATTAAAATA GCTTGCCCCC GTTGCAGCGA TGGGTATTTT
4551  TTCTAGTAAA ATAAAAGATA AACTTAGACT CAAAACATTT ACAAAAACAA CCCCTAAAGT CCTAAAGCCC
4621  AAAGTGCTAT GCACGATCCA TAGCAAGCCC AGCCCAACCC AACCCAACCC AACCCACCCC AGTGCAGCCA
4691  ACTGGCAAAT AGTCTCCACC CCCGGCACTA TCACCGTGAG TTGTCCGCAC CACCGCACGT CTCGCAGCCA
4761  AAAAAAAAAA AAGAAAGAAA AAAAAGAAAA AGAAAAACAG CAGGTGGGTC CGGGTCGTGG GGGCCGGAAA
4831  AGCGAGGAGG ATCGCGAGCA GCGACGAGGC CCGGCCCTCC CTCCGCTTCC AAAGAAACGC CCCCCATCGC
4901  CACTATATAC ATACCCCCCC CTCTCCTCCC ATCCCCCCAA CCCTACCACC ACCACCACCA CCACCTCCTC
4971  CCCCCTCGCT GCCGGACGAC GAGCTCCTCC CCCCTCCCCC TCCGCCGCCG CCGGTAACCA CCCCGCCCCT
5041  CTCCTCTTTC TTTCTCCGTT TTTTTTTTCG TCTCGGTCTC GATCTTTGGC CTTGGTAGTT TGGGTGGGCG
5111  AGAGCGGCTT CGTCGCCCAG ATCGGTGCGC GGGAGGGGCG GGATCTCGCG GCTGGCGTCT CCGGGCGTGA
              BamHI                              BglII
              ~~~~~~~~                           ~~~~~~~~
5181  GTCGGCCCGG ATCCTCGCGG GGAATGGGGC TCTCGGATGT AGATCTTCTT TCTTTCTTCT TTTTGTGGTA
5251  GAATTTGAAT CCCTCAGCAT TGTTCATCGG TAGTTTTTCT TTTCATGATT TGTGACAAAT GCAGCCTCGT
5321  GCGGAGCTTT TTTGTAGC
```

FIG._39E

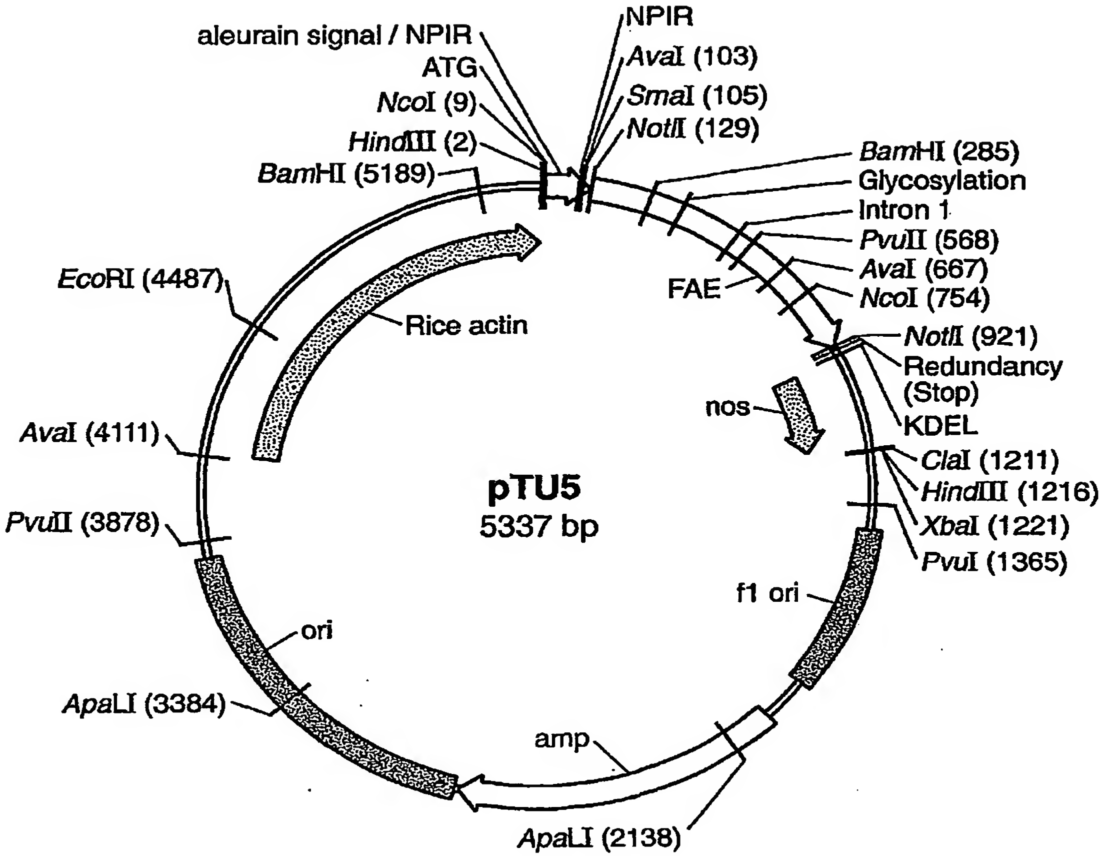

aleurain signal / NPIR
ATG
NcoI (9)
HindIII (2)
BamHI (5189)
NPIR
AvaI (103)
SmaI (105)
NotI (129)
BamHI (285)
Glycosylation
Intron 1
PvuII (568)
AvaI (667)
NcoI (754)
FAE
NotI (921)
Redundancy (Stop)
KDEL
nos
ClaI (1211)
HindIII (1216)
XbaI (1221)
PvuI (1365)
EcoRI (4487)
Rice actin
AvaI (4111)
PvuII (3878)
pTU5
5337 bp
f1 ori
ori
ApaLI (3384)
amp
ApaLI (2138)

FIG._40A

```
      HindIII NcoI
      ~~~~~~ ~~~~~~
   1  AAGCTTACCA TGGCCCACGC CCGCGTCCTC CTCCTGGCGC TCGCCGTGCT
      TTCGAATGGT ACCGGGTGCG GGCGCAGGAG GAGGACCGCG AGCGGCACGA

  51  GGCCACGGCC GCCGTCGCCG TCGCCTCCTC CTCCTCCTTC GCCGACTCCA
      CCGGTGCCGG CGGCAGCGGC AGCGGAGGAG GAGGAGGAAG CGGCTGAGGT

        SmaI
       ~~~~~~
        AvaI                       NotI
       ~~~~~~                     ~~~~~~~~~
 101  ACCCGGGCCG GCCCGTCACC GACCGCGCGG CCGCCTCCAC GCAGGGCATC
      TGGGCCCGGC CGGGCAGTGG CTGGCGCGCC GGCGGAGGTG CGTCCCGTAG

 151  TCCGAAGACC TCTACAGCCG TTTAGTCGAA ATGGCCACTA TCTCCCAAGC
      AGGCTTCTGG AGATGTCGGC AAATCAGCTT TACCGGTGAT AGAGGGTTCG

 201  TGCCTACGCC GACCTGTGCA ACATTCCGTC GACTATTATC AAGGGAGAGA
      ACGGATGCGG CTGGACACGT TGTAAGGCAG CTGATAATAG TTCCCTCTCT

                                           BamHI
                                          ~~~~~~
 251  AAATTTACAA TTCTCAAACT GACATTAACG GATGGATCCT CCGCGACGAC
      TTTAAATGTT AAGAGTTTGA CTGTAATTGC CTACCTAGGA GGCGCTGCTG

 301  AGCAGCAAAG AAATAATCAC CGTCTTCCGT GGCACTGGTA GTGATACGAA
      TCGTCGTTTC TTTATTAGTG GCAGAAGGCA CCGTGACCAT CACTATGCTT

 351  TCTACAACTC GATACTAACT ACACCCTCAC GCCTTTCGAC ACCCTACCAC
      AGATGTTGAG CTATGATTGA TGTGGGAGTG CGGAAAGCTG TGGGATGGTG

 401  AATGCAACGG TTGTGAAGTA CACGGTGGAT ATTATATTGG ATGGGTCTCC
      TTACGTTGCC AACACTTCAT GTGCCACCTA TAATATAACC TACCCAGAGG

 451  GTCCAGGACC AAGTCGAGTC GCTTGTCAAA CAGCAGGTTA GCCAGTATCC
      CAGGTCCTGG TTCAGCTCAG CGAACAGTTT GTCGTCCAAT CGGTCATAGG

 501  GGACTACGCG CTGACCGTGA CCGGCCACKC CCTCGGCGCC TCCCTGGCGG
      CCTGATGCGC GACTGGCACT GGCCGGTGMG GGAGCCGCGG AGGGACCGCC

                     PvuII
                     ~~~~~~
 551  CACTCACTGC CGCCCAGCTG TCTGCGACAT ACGACAACAT CCGCCTGTAC
      GTGAGTGACG GCGGGTCGAC AGACGCTGTA TGCTGTTGTA GGCGGACATG

 601  ACCTTCGGCG AACCGCGCAG CGGCAATCAG GCCTTCGCGT CGTACATGAA
      TGGAAGCCGC TTGGCGCGTC GCCGTTAGTC CGGAAGCGCA GCATGTACTT

                      AvaI
                     ~~~~~~~
 651  CGATGCCTTC CAAGCCTCGA GCCCAGATAC GACGCAGTAT TTCCGGGTCA
      GCTACGGAAG GTTCGGAGCT CGGGTCTATG CTGCGTCATA AAGGCCCAGT
```

FIG._40B

```
 701  CTCATGCCAA CGACGGCATC CCAAACCTGC CCCCGGTGGA GCAGGGGTAC
      GAGTACGGTT GCTGCCGTAG GGTTTGGACG GGGGCCACCT CGTCCCCATG

         NcoI
        ~~~~~~
 751  GCCCATGGCG GTGTAGAGTA CTGGAGCGTT GATCCTTACA GCGCCCAGAA
      CGGGTACCGC CACATCTCAT GACCTCGCAA CTAGGAATGT CGCGGGTCTT

 801  CACATTTGTC TGCACTGGGG ATGAAGTGCA GTGCTGTGAG GCCCAGGGCG
      GTGTAAACAG ACGTGACCCC TACTTCACGT CACGACACTC CGGGTCCCGC

 851  GACAGGGTGT GAATAATGCG CACACGACTT ATTTTGGGAT GACGAGCGGC
      CTGTCCCACA CTTATTACGC GTGTGCTGAA TAAAACCCTA CTGCTCGCCG

                            NotI
                         ~~~~~~~~~
 901  GCATGCACCT GGCCGGTCGC GGCCGCGGAA CCACTGAAGG ATGAGCTGTA
      CGTACGTGGA CCGGCCAGCG CCGGCGCCTT GGTGACTTCC TACTCGACAT

 951  AAGAAGCAGA TCGTTCAAAC ATTTGGCAAT AAAGTTTCTT AAGATTGAAT
      TTCTTCGTCT AGCAAGTTTG TAAACCGTTA TTTCAAAGAA TTCTAACTTA

1001  CCTGTTGCCG GTCTTGCGAT GATTATCATA TAATTTCTGT TGAATTACGT
      GGACAACGGC CAGAACGCTA CTAATAGTAT ATTAAAGACA ACTTAATGCA

1051  TAAGCATGTA ATAATTAACA TGTAATGCAT GACGTTATTT ATGAGATGGG
      ATTCGTACAT TATTAATTGT ACATTACGTA CTGCAATAAA TACTCTACCC

1101  TTTTTATGAT TAGAGTCCCG CAATTATACA TTTAATACGC GATAGAAAAC
      AAAAATACTA ATCTCAGGGC GTTAATATGT AAATTATGCG CTATCTTTTG

1151  AAAATATAGC GCGCAAACTA GGATAAATTA TCGCGCGCGG TGTCATCTAT
      TTTTATATCG CGCGTTTGAT CCTATTTAAT AGCGCGCGCC ACAGTAGATA

                           XbaI
                          ~~~~~~~
               ClaI   HindIII
              ~~~~~~~~~~~~~
1201  GTTACTAGAT CGATAAGCTT CTAGAGCGGC CGGTGGAGCT CCAATTCGCC
      CAATGATCTA GCTATTCGAA GATCTCGCCG GCCACCTCGA GGTTAAGCGG

1251  CTATAGTGAG TCGTATTACG CGCGCTCACT GGCCGTCGTT TTACAACGTC
      GATATCACTC AGCATAATGC GCGCGAGTGA CCGGCAGCAA AATGTTGCAG

1301  GTGACTGGGA AAACCCTGGC GTTACCCAAC TTAATCGCCT TGCAGCACAT
      CACTGACCCT TTTGGGACCG CAATGGGTTG AATTAGCGGA ACGTCGTGTA

                   PvuII
                  ~~~~~~
1351  CCCCCTTTCG CCAGCTGGCG TAATAGCGAA GAGGCCCGCA CCGATCGCCC
      GGGGGAAAGC GGTCGACCGC ATTATCGCTT CTCCGGGCGT GGCTAGCGGG

1401  TTCCCAACAG TTGCGCAGCC TGAATGGCGA ATGGGACGCG CCCTGTAGCG
      AAGGGTTGTC AACGCGTCGG ACTTACCGCT TACCCTGCGC GGGACATCGC
```

FIG._40C

```
1451  GCGCATTAAG CGCGGCGGGT GTGGTGGTTA CGCGCAGCGT GACCGCTACA
      CGCGTAATTC GCGCCGCCCA CACCACCAAT GCGCGTCGCA CTGGCGATGT

1501  CTTGCCAGCG CCCTAGCGCC CGCTCCTTTC GCTTTCTTCC CTTCCTTTCT
      GAACGGTCGC GGGATCGCGG GCGAGGAAAG CGAAAGAAGG GAAGGAAAGA

1551  CGCCACGTTC GCCGGCTTTC CCCGTCAAGC TCTAAATCGG GGGCTCCCTT
      GCGGTGCAAG CGGCCGAAAG GGGCAGTTCG AGATTTAGCC CCCGAGGGAA

1601  TAGGGTTCCG ATTTAGTGCT TTACGGCACC TCGACCCCAA AAAACTTGAT
      ATCCCAAGGC TAAATCACGA AATGCCGTGG AGCTGGGGTT TTTTGAACTA

1651  TAGGGTGATG GTTCACGTAG TGGGCCATCG CCCTGATAGA CGGTTTTTCG
      ATCCCACTAC CAAGTGCATC ACCCGGTAGC GGGACTATCT GCCAAAAAGC

1701  CCCTTTGACG TTGGAGTCCA CGTTCTTTAA TAGTGGACTC TTGTTCCAAA
      GGGAAACTGC AACCTCAGGT GCAAGAAATT ATCACCTGAG AACAAGGTTT

1751  CTGGAACAAC ACTCAACCCT ATCTCGGTCT ATTCTTTTGA TTTATAAGGG
      GACCTTGTTG TGAGTTGGGA TAGAGCCAGA TAAGAAAACT AAATATTCCC

1801  ATTTTGCCGA TTTCGGCCTA TTGGTTAAAA AATGAGCTGA TTTAACAAAA
      TAAAACGGCT AAAGCCGGAT AACCAATTTT TTACTCGACT AAATTGTTTT

1851  ATTTAACGCG AATTTTAACA AAATATTAAC GCTTACAATT TAGGTGGCAC
      TAAATTGCGC TTAAAATTGT TTTATAATTG CGAATGTTAA ATCCACCGTG

1901  TTTTCGGGGA AATGTGCGCG GAACCCCTAT TTGTTTATTT TTCTAAATAC
      AAAAGCCCCT TTACACGCGC CTTGGGGATA AACAAATAAA AAGATTTATG

1951  ATTCAAATAT GTATCCGCTC ATGAGACAAT AACCCTGATA AATGCTTCAA
      TAAGTTTATA CATAGGCGAG TACTCTGTTA TTGGGACTAT TTACGAAGTT

2001  TAATATTGAA AAAGGAAGAG TATGAGTATT CAACATTTCC GTGTCGCCCT
      ATTATAACTT TTTCCTTCTC ATACTCATAA GTTGTAAAGG CACAGCGGGA

2051  TATTCCCTTT TTTGCGGCAT TTTGCCTTCC TGTTTTTGCT CACCCAGAAA
      ATAAGGGAAA AAACGCCGTA AAACGGAAGG ACAAAAACGA GTGGGTCTTT

                                              ApaLI
                                             ~~~~~~~
2101  CGCTGGTGAA AGTAAAAGAT GCTGAAGATC AGTTGGGTGC ACGAGTGGGT
      GCGACCACTT TCATTTTCTA CGACTTCTAG TCAACCCACG TGCTCACCCA

2151  TACATCGAAC TGGATCTCAA CAGCGGTAAG ATCCTTGAGA GTTTTCGCCC
      ATGTAGCTTG ACCTAGAGTT GTCGCCATTC TAGGAACTCT CAAAAGCGGG

2201  CGAAGAACGT TTTCCAATGA TGAGCACTTT TAAAGTTCTG CTATGTGGCG
      GCTTCTTGCA AAAGGTTACT ACTCGTGAAA ATTTCAAGAC GATACACCGC

2251  CGGTATTATC CCGTATTGAC GCCGGGCAAG AGCAACTCGG TCGCCGCATA
      GCCATAATAG GGCATAACTG CGGCCCGTTC TCGTTGAGCC AGCGGCGTAT

2301  CACTATTCTC AGAATGACTT GGTTGAGTAC TCACCAGTCA CAGAAAAGCA
      GTGATAAGAG TCTTACTGAA CCAACTCATG AGTGGTCAGT GTCTTTTCGT
```

FIG._40D

```
2351  TCTTACGGAT GGCATGACAG TAAGAGAATT ATGCAGTGCT GCCATAACCA
      AGAATGCCTA CCGTACTGTC ATTCTCTTAA TACGTCACGA CGGTATTGGT

2401  TGAGTGATAA CACTGCGGCC AACTTACTTC TGACAACGAT CGGAGGACCG
      ACTCACTATT GTGACGCCGG TTGAATGAAG ACTGTTGCTA GCCTCCTGGC

2451  AAGGAGCTAA CCGCTTTTTT GCACAACATG GGGGATCATG TAACTCGCCT
      TTCCTCGATT GGCGAAAAAA CGTGTTGTAC CCCCTAGTAC ATTGAGCGGA

2501  TGATCGTTGG GAACCGGAGC TGAATGAAGC CATACCAAAC GACGAGCGTG
      ACTAGCAACC CTTGGCCTCG ACTTACTTCG GTATGGTTTG CTGCTCGCAC

2551  ACACCACGAT GCCTGTAGCA ATGGCAACAA CGTTGCGCAA ACTATTAACT
      TGTGGTGCTA CGGACATCGT TACCGTTGTT GCAACGCGTT TGATAATTGA

2601  GGCGAACTAC TTACTCTAGC TTCCCGGCAA CAATTAATAG ACTGGATGGA
      CCGCTTGATG AATGAGATCG AAGGGCCGTT GTTAATTATC TGACCTACCT

2651  GGCGGATAAA GTTGCAGGAC CACTTCTGCG CTCGGCCCTT CCGGCTGGCT
      CCGCCTATTT CAACGTCCTG GTGAAGACGC GAGCCGGGAA GGCCGACCGA

2701  GGTTTATTGC TGATAAATCT GGAGCCGGTG AGCGTGGGTC TCGCGGTATC
      CCAAATAACG ACTATTTAGA CCTCGGCCAC TCGCACCCAG AGCGCCATAG

2751  ATTGCAGCAC TGGGGCCAGA TGGTAAGCCC TCCCGTATCG TAGTTATCTA
      TAACGTCGTG ACCCCGGTCT ACCATTCGGG AGGGCATAGC ATCAATAGAT

2801  CACGACGGGG AGTCAGGCAA CTATGGATGA ACGAAATAGA CAGATCGCTG
      GTGCTGCCCC TCAGTCCGTT GATACCTACT TGCTTTATCT GTCTAGCGAC

2851  AGATAGGTGC CTCACTGATT AAGCATTGGT AACTGTCAGA CCAAGTTTAC
      TCTATCCACG GAGTGACTAA TTCGTAACCA TTGACAGTCT GGTTCAAATG

2901  TCATATATAC TTTAGATTGA TTTAAAACTT CATTTTTAAT TTAAAAGGAT
      AGTATATATG AAATCTAACT AAATTTTGAA GTAAAAATTA AATTTTCCTA

2951  CTAGGTGAAG ATCCTTTTTG ATAATCTCAT GACCAAAATC CCTTAACGTG
      GATCCACTTC TAGGAAAAAC TATTAGAGTA CTGGTTTTAG GGAATTGCAC

3001  AGTTTTCGTT CCACTGAGCG TCAGACCCCG TAGAAAAGAT CAAAGGATCT
      TCAAAAGCAA GGTGACTCGC AGTCTGGGGC ATCTTTTCTA GTTTCCTAGA

3051  TCTTGAGATC CTTTTTTTCT GCGCGTAATC TGCTGCTTGC AAACAAAAAA
      AGAACTCTAG GAAAAAAAGA CGCGCATTAG ACGACGAACG TTTGTTTTTT

3101  ACCACCGCTA CCAGCGGTGG TTTGTTTGCC GGATCAAGAG CTACCAACTC
      TGGTGGCGAT GGTCGCCACC AAACAAACGG CCTAGTTCTC GATGGTTGAG

3151  TTTTTCCGAA GGTAACTGGC TTCAGCAGAG CGCAGATACC AAATACTGTC
      AAAAAGGCTT CCATTGACCG AAGTCGTCTC GCGTCTATGG TTTATGACAG

3201  CTTCTAGTGT AGCCGTAGTT AGGCCACCAC TTCAAGAACT CTGTAGCACC
      GAAGATCACA TCGGCATCAA TCCGGTGGTG AAGTTCTTGA GACATCGTGG

3251  GCCTACATAC CTCGCTCTGC TAATCCTGTT ACCAGTGGCT GCTGCCAGTG
      CGGATGTATG GAGCGAGACG ATTAGGACAA TGGTCACCGA CGACGGTCAC
```

FIG._40E

```
3301  GCGATAAGTC GTGTCTTACC GGGTTGGACT CAAGACGATA GTTACCGGAT
      CGCTATTCAG CACAGAATGG CCCAACCTGA GTTCTGCTAT CAATGGCCTA

                                          ApaLI
                                         ~~~~~~
3351  AAGGCGCAGC GGTCGGGCTG AACGGGGGGT TCGTGCACAC AGCCCAGCTT
      TTCCGCGTCG CCAGCCCGAC TTGCCCCCCA AGCACGTGTG TCGGGTCGAA

3401  GGAGCGAACG ACCTACACCG AACTGAGATA CCTACAGCGT GAGCTATGAG
      CCTCGCTTGC TGGATGTGGC TTGACTCTAT GGATGTCGCA CTCGATACTC

3451  AAAGCGCCAC GCTTCCCGAA GGGAGAAAGG CGGACAGGTA TCCGGTAAGC
      TTTCGCGGTG CGAAGGGCTT CCCTCTTTCC GCCTGTCCAT AGGCCATTCG

3501  GGCAGGGTCG GAACAGGAGA GCGCACGAGG GAGCTTCCAG GGGGAAACGC
      CCGTCCCAGC CTTGTCCTCT CGCGTGCTCC CTCGAAGGTC CCCCTTTGCG

3551  CTGGTATCTT TATAGTCCTG TCGGGTTTCG CCACCTCTGA CTTGAGCGTC
      GACCATAGAA ATATCAGGAC AGCCCAAAGC GGTGGAGACT GAACTCGCAG

3601  GATTTTTGTG ATGCTCGTCA GGGGGGCGGA GCCTATGGAA AAACGCCAGC
      CTAAAAACAC TACGAGCAGT CCCCCCGCCT CGGATACCTT TTTGCGGTCG

3651  AACGCGGCCT TTTTACGGTT CCTGGCCTTT TGCTGGCCTT TTGCTCACAT
      TTGCGCCGGA AAAATGCCAA GGACCGGAAA ACGACCGGAA AACGAGTGTA

3701  GTTCTTTCCT GCGTTATCCC CTGATTCTGT GGATAACCGT ATTACCGCCT
      CAAGAAAGGA CGCAATAGGG GACTAAGACA CCTATTGGCA TAATGGCGGA

3751  TTGAGTGAGC TGATACCGCT CGCCGCAGCC GAACGACCGA GCGCAGCGAG
      AACTCACTCG ACTATGGCGA GCGGCGTCGG CTTGCTGGCT CGCGTCGCTC

3801  TCAGTGAGCG AGGAAGCGGA AGAGCGCCCA ATACGCAAAC CGCCTCTCCC
      AGTCACTCGC TCCTTCGCCT TCTCGCGGGT TATGCGTTTG GCGGAGAGGG

                                 PvuII
                                ~~~~~~
3851  CGCGCGTTGG CCGATTCATT AATGCAGCTG GCACGACAGG TTTCCCGACT
      GCGCGCAACC GGCTAAGTAA TTACGTCGAC CGTGCTGTCC AAAGGGCTGA

3901  GGAAAGCGGG CAGTGAGCGC AACGCAATTA ATGTGAGTTA GCTCACTCAT
      CCTTTCGCCC GTCACTCGCG TTGCGTTAAT TACACTCAAT CGAGTGAGTA

3951  TAGGCACCCC AGGCTTTACA CTTTATGCTT CCGGCTCGTA TGTTGTGTGG
      ATCCGTGGGG TCCGAAATGT GAAATACGAA GGCCGAGCAT ACAACACACC

4001  AATTGTGAGC GGATAACAAT TTCACACAGG AAACAGCTAT GACCATGATT
      TTAACACTCG CCTATTGTTA AAGTGTGTCC TTTGTCGATA CTGGTACTAA

4051  ACGCCAAGCG CGCAATTAAC CCTCACTAAA GGGAACAAAA GCTGGGTACC
      TGCGGTTCGC GCGTTAATTG GGAGTGATTT CCCTTGTTTT CGACCCATGG

                  AvaI
                ~~~~~~~
4101  GGGCCCCCCC TCGAGGTCAT TCATATGCTT GAGAAGAGAG TCGGGATAGT
      CCCGGGGGGG AGCTCCAGTA AGTATACGAA CTCTTCTCTC AGCCCTATCA
```

FIG._40F

```
4151    CCAAAATAAA ACAAAGGTAA GATTACCTGG TCAAAAGTGA AAACATCAGT
        GGTTTTATTT TGTTTCCATT CTAATGGACC AGTTTTCACT TTTGTAGTCA

4201    TAAAAGGTGG TATAAGTAAA ATATCGGTAA TAAAAGGTGG CCCAAAGTGA
        ATTTTCCACC ATATTCATTT TATAGCCATT ATTTTCCACC GGGTTTCACT

4251    AATTTACTCT TTTCTACTAT TATAAAAATT GAGGATGTTT TGTCGGTACT
        TTAAATGAGA AAAGATGATA ATATTTTTAA CTCCTACAAA ACAGCCATGA

4301    TTGATACGTC ATTTTTGTAT GAATTGGTTT TTAAGTTTAT TCGCGATTTG
        AACTATGCAG TAAAAACATA CTTAACCAAA AATTCAAATA AGCGCTAAAC

4351    GAAATGCATA TCTGTATTTG AGTCGGTTTT TAAGTTCGTT GCTTTTGTAA
        CTTTACGTAT AGACATAAAC TCAGCCAAAA ATTCAAGCAA CGAAAACATT

4401    ATACAGAGGG ATTTGTATAA GAAATATCTT TAAAAAACCC ATATGCTAAT
        TATGTCTCCC TAAACATATT CTTTATAGAA ATTTTTTGGG TATACGATTA

                                              EcoRI
                                              ~~~~~~~
4451    TTGACATAAT TTTTGAGAAA AATATATATT CAGGCGAATT CCACAATGAA
        AACTGTATTA AAAACTCTTT TTATATATAA GTCCGCTTAA GGTGTTACTT

4501    CAATAATAAG ATTAAAATAG CTTGCCCCCG TTGCAGCGAT GGGTATTTTT
        GTTATTATTC TAATTTTATC GAACGGGGGC AACGTCGCTA CCCATAAAAA

4551    TCTAGTAAAA TAAAAGATAA ACTTAGACTC AAAACATTTA CAAAAACAAC
        AGATCATTTT ATTTTCTATT TGAATCTGAG TTTTGTAAAT GTTTTTGTTG

4601    CCCTAAAGTC CTAAAGCCCA AAGTGCTATG CACGATCCAT AGCAAGCCCA
        GGGATTTCAG GATTTCGGGT TTCACGATAC GTGCTAGGTA TCGTTCGGGT

4651    GCCCAACCCA ACCCAACCCA ACCCACCCCA GTGCAGCCAA CTGGCAAATA
        CGGGTTGGGT TGGGTTGGGT TGGGTGGGGT CACGTCGGTT GACCGTTTAT

4701    GTCTCCACCC CCGGCACTAT CACCGTGAGT TGTCCGCACC ACCGCACGTC
        CAGAGGTGGG GGCCGTGATA GTGGCACTCA ACAGGCGTGG TGGCGTGCAG

4751    TCGCAGCCAA AAAAAAAAAA AGAAAGAAAA AAAAGAAAAA GAAAAACAGC
        AGCGTCGGTT TTTTTTTTTT TCTTTCTTTT TTTTCTTTTT CTTTTTGTCG

4801    AGGTGGGTCC GGGTCGTGGG GGCCGGAAAA GCGAGGAGGA TCGCGAGCAG
        TCCACCCAGG CCCAGCACCC CCGGCCTTTT CGCTCCTCCT AGCGCTCGTC

4851    CGACGAGGCC CGGCCCTCCC TCCGCTTCCA AAGAAACGCC CCCCATCGCC
        GCTGCTCCGG GCCGGGAGGG AGGCGAAGGT TTCTTTGCGG GGGGTAGCGG

4901    ACTATATACA TACCCCCCCC TCTCCTCCCA TCCCCCCAAC CCTACCACCA
        TGATATATGT ATGGGGGGGG AGAGGAGGGT AGGGGGGTTG GGATGGTGGT

4951    CCACCACCAC CACCTCCTCC CCCCTCGCTG CCGGACGACG AGCTCCTCCC
        GGTGGTGGTG GTGGAGGAGG GGGGAGCGAC GGCCTGCTGC TCGAGGAGGG

5001    CCCTCCCCCT CCGCCGCCGC CGGTAACCAC CCCGCCCCTC TCCTCTTTCT
        GGGAGGGGGA GGCGGCGGCG GCCATTGGTG GGGCGGGGAG AGGAGAAAGA
```

FIG._40G

```
5051  TTCTCCGTTT TTTTTTTCGT CTCGGTCTCG ATCTTTGGCC TTGGTAGTTT
      AAGAGGCAAA AAAAAAAGCA GAGCCAGAGC TAGAAACCGG AACCATCAAA

5101  GGGTGGGCGA GAGCGGCTTC GTCGCCCAGA TCGGTGCGCG GGAGGGGCGG
      CCCACCCGCT CTCGCCGAAG CAGCGGGTCT AGCCACGCGC CCTCCCCGCC

                                                BamHI
                                              ~~~~~~~
5151  GATCTCGCGG CTGGCGTCTC CGGGCGTGAG TCGGCCCGGA TCCTCGCGGG
      CTAGAGCGCC GACCGCAGAG GCCCGCACTC AGCCGGGCCT AGGAGCGCCC

5201  GAATGGGGCT CTCGGATGTA GATCTTCTTT CTTTCTTCTT TTTGTGGTAG
      CTTACCCCGA GAGCCTACAT CTAGAAGAAA GAAAGAAGAA AAACACCATC

5251  AATTTGAATC CCTCAGCATT GTTCATCGGT AGTTTTTCTT TTCATGATTT
      TTAAACTTAG GGAGTCGTAA CAAGTAGCCA TCAAAAAGAA AAGTACTAAA

5301  GTGACAAATG CAGCCTCGTG CGGAGCTTTT TTGTAGC
      CACTGTTTAC GTCGGAGCAC GCCTCGAAAA AACATCG
```

FIG._40H

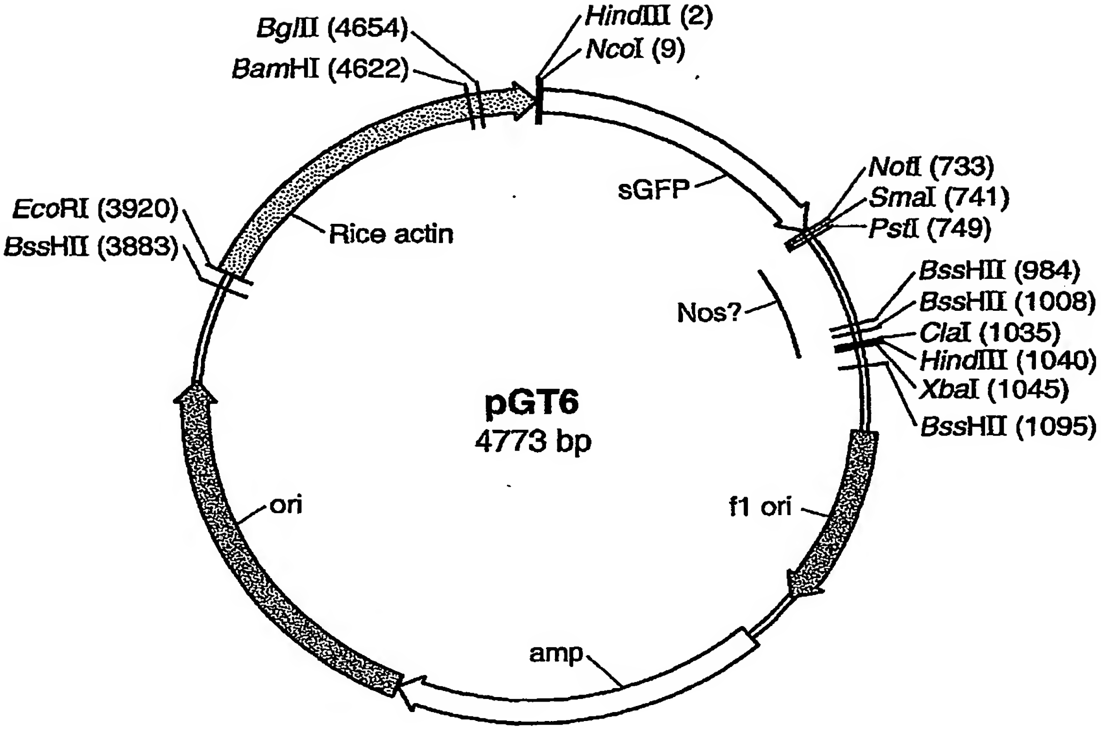

BglII (4654)
BamHI (4622)
HindIII (2)
NcoI (9)
sGFP
NotI (733)
SmaI (741)
PstI (749)
EcoRI (3920)
BssHII (3883)
Rice actin
Nos?
BssHII (984)
BssHII (1008)
ClaI (1035)
HindIII (1040)
XbaI (1045)
BssHII (1095)
pGT6
4773 bp
ori
f1 ori
amp

FIG._41A

```
     HindIII NcoI
     ~~~~~~~ ~~~~~~~~
  1  AAGCTTACCA TGGTGAGCAA GGGCGAGGAG CTGTTCACCG GGGTGGTGCC CATCCTGGTC GAGCTGGACG
     TTCGAATGGT ACCACTCGTT CCCGCTCCTC GACAAGTGGC CCCACCACGG GTAGGACCAG CTCGACCTGC

 71  GCGACGTGAA CGGCCACAAG TTCAGCGTGT CCGGCGAGGG CGAGGGCGAT GCCACCTACG GCAAGCTGAC
     CGCTGCACTT GCCGGTGTTC AAGTCGCACA GGCCGCTCCC GCTCCCGCTA CGGTGGATGC CGTTCGACTG

141  CCTGAAGTTC ATCTGCACCA CCGGCAAGCT GCCCGTGCCC TGGCCCACCC TCGTGACCAC CTTCACCTAC
     GGACTTCAAG TAGACGTGGT GGCCGTTCGA CGGGCACGGG ACCGGGTGGG AGCACTGGTG GAAGTGGATG

211  GGCGTGCAGT GCTTCAGCCG CTACCCCGAC CACATGAAGC AGCACGACTT CTTCAAGTCC GCCATGCCCG
     CCGCACGTCA CGAAGTCGGC GATGGGGCTG GTGTACTTCG TCGTGCTGAA GAAGTTCAGG CGGTACGGGC

281  AAGGCTACGT CCAGGAGCGC ACCATCTTCT TCAAGGACGA CGGCAACTAC AAGACCCGCG CCGAGGTGAA
     TTCCGATGCA GGTCCTCGCG TGGTAGAAGA AGTTCCTGCT GCCGTTGATG TTCTGGGCGC GGCTCCACTT

351  GTTCGAGGGC GACACCCTGG TGAACCGCAT CGAGCTGAAG GGCATCGACT TCAAGGAGGA CGGCAACATC
     CAAGCTCCCG CTGTGGGACC ACTTGGCGTA GCTCGACTTC CCGTAGCTGA AGTTCCTCCT GCCGTTGTAG

421  CTGGGGCACA AGCTGGAGTA CAACTACAAC AGCCACAACG TCTATATCAT GGCCGACAAG CAGAAGAACG
     GACCCCGTGT TCGACCTCAT GTTGATGTTG TCGGTGTTGC AGATATAGTA CCGGCTGTTC GTCTTCTTGC

491  GCATCAAGGT GAACTTCAAG ATCCGCCACA ACATCGAGGA CGGCAGCGTG CAGCTCGCCG ACCACTACCA
     CGTAGTTCCA CTTGAAGTTC TAGGCGGTGT TGTAGCTCCT GCCGTCGCAC GTCGAGCGGC TGGTGATGGT

561  GCAGAACACC CCCATCGGCG ACGGCCCCGT GCTGCTGCCC GACAACCACT ACCTGAGCAC CCAGTCCGCC
     CGTCTTGTGG GGGTAGCCGC TGCCGGGGCA CGACGACGGG CTGTTGGTGA TGGACTCGTG GGTCAGGCGG

631  CTGAGCAAAG ACCCCAACGA GAAGCGCGAT CACATGGTCC TGCTGGAGTT CGTGACCGCC GCCGGGATCA
     GACTCGTTTC TGGGGTTGCT CTTCGCGCTA GTGTACCAGG ACGACCTCAA GCACTGGCGG CGGCCCTAGT
```

FIG._41B

```
                                                SmaI
                                               ~~~~~~~
                                          NotI         PstI
                                        ~~~~~~~~      ~~~~~~
  701  CTCACGGCAT GGACGAGCTG TACAAGTAAA GCGGCCGCCC GGGCTGCAGG GAAACCACTG AAGGATGAGC
       GAGTGCCGTA CCTGCTCGAC ATGTTCATTT CGCCGGCGGG CCCGACGTCC CTTTGGTGAC TTCCTACTCG

  771  TGTAAAGAAG CAGATCGTTC AAACATTTGG CAATAAAGTT TCTTAAGATT GAATCCTGTT GCCGGTCTTG
       ACATTTCTTC GTCTAGCAAG TTTGTAAACC GTTATTTCAA AGAATTCTAA CTTAGGACAA CGGCCAGAAC

  841  CGATGATTAT CATATAATTT CTGTTGAATT ACGTTAAGCA TGTAATAATT AACATGTAAT GCATGACGTT
       GCTACTAATA GTATATTAAA GACAACTTAA TGCAATTCGT ACATTATTAA TTGTACATTA CGTACTGCAA

  911  ATTTATGAGA TGGGTTTTTA TGATTAGAGT CCCGCAATTA TACATTTAAT ACGCGATAGA AAACAAAATA
       TAAATACTCT ACCCAAAAAT ACTAATCTCA GGGCGTTAAT ATGTAAATTA TGCGCTATCT TTTGTTTTAT

                                                                              XbaI
                                                                            ~~~~~~
         BssHII                BssHII                      ClaI HindIII
         ~~~~~~                    ~~~~~~~                      ~~~~~~~~~~~~~
  981  TAGCGCGCAA ACTAGGATAA ATTATCGCGC GCGGTGTCAT CTATGTTACT AGATCGATAA GCTTCTAGAG
       ATCGCGCGTT TGATCCTATT TAATAGCGCG CGCCACAGTA GATACAATGA TCTAGCTATT CGAAGATCTC

                                                      BssHII
                                                      ~~~~~~
 1051  CGGCCGGTGG AGCTCCAATT CGCCCTATAG TGAGTCGTAT TACGCGCGCT CACTGGCCGT CGTTTTACAA
       GCCGGCCACC TCGAGGTTAA GCGGGATATC ACTCAGCATA ATGCGCGCGA GTGACCGGCA GCAAAATGTT

 1121  CGTCGTGACT GGGAAAACCC TGGCGTTACC CAACTTAATC GCCTTGCAGC ACATCCCCCT TTCGCCAGCT
       GCAGCACTGA CCCTTTTGGG ACCGCAATGG GTTGAATTAG CGGAACGTCG TGTAGGGGGA AAGCGGTCGA

 1191  GGCGTAATAG CGAAGAGGCC CGCACCGATC GCCCTTCCCA ACAGTTGCGC AGCCTGAATG GCGAATGGGA
       CCGCATTATC GCTTCTCCGG GCGTGGCTAG CGGGAAGGGT TGTCAACGCG TCGGACTTAC CGCTTACCCT

 1261  CGCGCCCTGT AGCGGCGCAT TAAGCGCGGC GGGTGTGGTG GTTACGCGCA GCGTGACCGC TACACTTGCC
       GCGCGGGACA TCGCCGCGTA ATTCGCGCCG CCCACACCAC CAATGCGCGT CGCACTGGCG ATGTGAACGG
```

FIG._41C

```
1331  AGCGCCCTAG CGCCCGCTCC TTTCGCTTTC TTCCCTTCCT TTCTCGCCAC GTTCGCCGGC TTTCCCCGTC
      TCGCGGGATC GCGGGCGAGG AAAGCGAAAG AAGGGAAGGA AAGAGCGGTG CAAGCGGCCG AAAGGGGCAG

1401  AAGCTCTAAA TCGGGGGCTC CCTTTAGGGT TCCGATTTAG TGCTTTACGG CACCTCGACC CCAAAAAACT
      TTCGAGATTT AGCCCCCGAG GGAAATCCCA AGGCTAAATC ACGAAATGCC GTGGAGCTGG GGTTTTTTGA

1471  TGATTAGGGT GATGGTTCAC GTAGTGGGCC ATCGCCCTGA TAGACGGTTT TTCGCCCTTT GACGTTGGAG
      ACTAATCCCA CTACCAAGTG CATCACCCGG TAGCGGGACT ATCTGCCAAA AAGCGGGAAA CTGCAACCTC

1541  TCCACGTTCT TTAATAGTGG ACTCTTGTTC CAAACTGGAA CAACACTCAA CCCTATCTCG GTCTATTCTT
      AGGTGCAAGA AATTATCACC TGAGAACAAG GTTTGACCTT GTTGTGAGTT GGGATAGAGC CAGATAAGAA

1611  TTGATTTATA AGGGATTTTG CCGATTTCGG CCTATTGGTT AAAAAATGAG CTGATTTAAC AAAAATTTAA
      AACTAAATAT TCCCTAAAAC GGCTAAAGCC GGATAACCAA TTTTTTACTC GACTAAATTG TTTTTAAATT

1681  CGCGAATTTT AACAAAATAT TAACGCTTAC AATTTAGGTG GCACTTTTCG GGGAAATGTG CGCGGAACCC
      GCGCTTAAAA TTGTTTTATA ATTGCGAATG TTAAATCCAC CGTGAAAAGC CCCTTTACAC GCGCCTTGGG

1751  CTATTTGTTT ATTTTTCTAA ATACATTCAA ATATGTATCC GCTCATGAGA CAATAACCCT GATAAATGCT
      GATAAACAAA TAAAAAGATT TATGTAAGTT TATACATAGG CGAGTACTCT GTTATTGGGA CTATTTACGA

1821  TCAATAATAT TGAAAAAGGA AGAGTATGAG TATTCAACAT TTCCGTGTCG CCCTTATTCC CTTTTTTGCG
      AGTTATTATA ACTTTTTCCT TCTCATACTC ATAAGTTGTA AAGGCACAGC GGGAATAAGG GAAAAAACGC

1891  GCATTTTGCC TTCCTGTTTT TGCTCACCCA GAAACGCTGG TGAAAGTAAA AGATGCTGAA GATCAGTTGG
      CGTAAAACGG AAGGACAAAA ACGAGTGGGT CTTTGCGACC ACTTTCATTT TCTACGACTT CTAGTCAACC

1961  GTGCACGAGT GGGTTACATC GAACTGGATC TCAACAGCGG TAAGATCCTT GAGAGTTTTC GCCCCGAAGA
      CACGTGCTCA CCCAATGTAG CTTGACCTAG AGTTGTCGCC ATTCTAGGAA CTCTCAAAAG CGGGGCTTCT

2031  ACGTTTTCCA ATGATGAGCA CTTTTAAAGT TCTGCTATGT GGCGCGGTAT TATCCCGTAT TGACGCCGGG
      TGCAAAAGGT TACTACTCGT GAAAATTTCA AGACGATACA CCGCGCCATA ATAGGGCATA ACTGCGGCCC

2101  CAAGAGCAAC TCGGTCGCCG CATACACTAT TCTCAGAATG ACTTGGTTGA GTACTCACCA GTCACAGAAA
      GTTCTCGTTG AGCCAGCGGC GTATGTGATA AGAGTCTTAC TGAACCAACT CATGAGTGGT CAGTGTCTTT
```

FIG._41D

```
2171  AGCATCTTAC GGATGGCATG ACAGTAAGAG AATTATGCAG TGCTGCCATA ACCATGAGTG ATAACACTGC
      TCGTAGAATG CCTACCGTAC TGTCATTCTC TTAATACGTC ACGACGGTAT TGGTACTCAC TATTGTGACG

2241  GGCCAACTTA CTTCTGACAA CGATCGGAGG ACCGAAGGAG CTAACCGCTT TTTTGCACAA CATGGGGGAT
      CCGGTTGAAT GAAGACTGTT GCTAGCCTCC TGGCTTCCTC GATTGGCGAA AAAACGTGTT GTACCCCCTA

2311  CATGTAACTC GCCTTGATCG TTGGGAACCG GAGCTGAATG AAGCCATACC AAACGACGAG CGTGACACCA
      GTACATTGAG CGGAACTAGC AACCCTTGGC CTCGACTTAC TTCGGTATGG TTTGCTGCTC GCACTGTGGT

2381  CGATGCCTGT AGCAATGGCA ACAACGTTGC GCAAACTATT AACTGGCGAA CTACTTACTC TAGCTTCCCG
      GCTACGGACA TCGTTACCGT TGTTGCAACG CGTTTGATAA TTGACCGCTT GATGAATGAG ATCGAAGGGC

2451  GCAACAATTA ATAGACTGGA TGGAGGCGGA TAAAGTTGCA GGACCACTTC TGCGCTCGGC CCTTCCGGCT
      CGTTGTTAAT TATCTGACCT ACCTCCGCCT ATTTCAACGT CCTGGTGAAG ACGCGAGCCG GGAAGGCCGA

2521  GGCTGGTTTA TTGCTGATAA ATCTGGAGCC GGTGAGCGTG GGTCTCGCGG TATCATTGCA GCACTGGGGC
      CCGACCAAAT AACGACTATT TAGACCTCGG CCACTCGCAC CCAGAGCGCC ATAGTAACGT CGTGACCCCG

2591  CAGATGGTAA GCCCTCCCGT ATCGTAGTTA TCTACACGAC GGGGAGTCAG GCAACTATGG ATGAACGAAA
      GTCTACCATT CGGGAGGGCA TAGCATCAAT AGATGTGCTG CCCCTCAGTC CGTTGATACC TACTTGCTTT

2661  TAGACAGATC GCTGAGATAG GTGCCTCACT GATTAAGCAT TGGTAACTGT CAGACCAAGT TTACTCATAT
      ATCTGTCTAG CGACTCTATC CACGGAGTGA CTAATTCGTA ACCATTGACA GTCTGGTTCA AATGAGTATA

2731  ATACTTTAGA TTGATTTAAA ACTTCATTTT TAATTTAAAA GGATCTAGGT GAAGATCCTT TTTGATAATC
      TATGAAATCT AACTAAATTT TGAAGTAAAA ATTAAATTTT CCTAGATCCA CTTCTAGGAA AAACTATTAG

2801  TCATGACCAA AATCCCTTAA CGTGAGTTTT CGTTCCACTG AGCGTCAGAC CCCGTAGAAA AGATCAAAGG
      AGTACTGGTT TTAGGGAATT GCACTCAAAA GCAAGGTGAC TCGCAGTCTG GGGCATCTTT TCTAGTTTCC

2871  ATCTTCTTGA GATCCTTTTT TTCTGCGCGT AATCTGCTGC TTGCAAACAA AAAAACCACC GCTACCAGCG
      TAGAAGAACT CTAGGAAAAA AAGACGCGCA TTAGACGACG AACGTTTGTT TTTTTGGTGG CGATGGTCGC

2941  GTGGTTTGTT TGCCGGATCA AGAGCTACCA ACTCTTTTTC CGAAGGTAAC TGGCTTCAGC AGAGCGCAGA
      CACCAAACAA ACGGCCTAGT TCTCGATGGT TGAGAAAAAG GCTTCCATTG ACCGAAGTCG TCTCGCGTCT
```

FIG._41E

```
3011  TACCAAATAC TGTCCTTCTA GTGTAGCCGT AGTTAGGCCA CCACTTCAAG AACTCTGTAG CACCGCCTAC
      ATGGTTTATG ACAGGAAGAT CACATCGGCA TCAATCCGGT GGTGAAGTTC TTGAGACATC GTGGCGGATG

3081  ATACCTCGCT CTGCTAATCC TGTTACCAGT GGCTGCTGCC AGTGGCGATA AGTCGTGTCT TACCGGGTTG
      TATGGAGCGA GACGATTAGG ACAATGGTCA CCGACGACGG TCACCGCTAT TCAGCACAGA ATGGCCCAAC

3151  GACTCAAGAC GATAGTTACC GGATAAGGCG CAGCGGTCGG GCTGAACGGG GGGTTCGTGC ACACAGCCCA
      CTGAGTTCTG CTATCAATGG CCTATTCCGC GTCGCCAGCC CGACTTGCCC CCCAAGCACG TGTGTCGGGT

3221  GCTTGGAGCG AACGACCTAC ACCGAACTGA GATACCTACA GCGTGAGCTA TGAGAAAGCG CCACGCTTCC
      CGAACCTCGC TTGCTGGATG TGGCTTGACT CTATGGATGT CGCACTCGAT ACTCTTTCGC GGTGCGAAGG

3291  CGAAGGGAGA AAGGCGGACA GGTATCCGGT AAGCGGCAGG GTCGGAACAG GAGAGCGCAC GAGGGAGCTT
      GCTTCCCTCT TTCCGCCTGT CCATAGGCCA TTCGCCGTCC CAGCCTTGTC CTCTCGCGTG CTCCCTCGAA

3361  CCAGGGGGAA ACGCCTGGTA TCTTTATAGT CCTGTCGGGT TTCGCCACCT CTGACTTGAG CGTCGATTTT
      GGTCCCCCTT TGCGGACCAT AGAAATATCA GGACAGCCCA AAGCGGTGGA GACTGAACTC GCAGCTAAAA

3431  TGTGATGCTC GTCAGGGGGG CGGAGCCTAT GGAAAAACGC CAGCAACGCG GCCTTTTTAC GGTTCCTGGC
      ACACTACGAG CAGTCCCCCC GCCTCGGATA CCTTTTTGCG GTCGTTGCGC CGGAAAAATG CCAAGGACCG

3501  CTTTTGCTGG CCTTTTGCTC ACATGTTCTT TCCTGCGTTA TCCCCTGATT CTGTGGATAA CCGTATTACC
      GAAAACGACC GGAAAACGAG TGTACAAGAA AGGACGCAAT AGGGGACTAA GACACCTATT GGCATAATGG

3571  GCCTTTGAGT GAGCTGATAC CGCTCGCCGC AGCCGAACGA CCGAGCGCAG CGAGTCAGTG AGCGAGGAAG
      CGGAAACTCA CTCGACTATG GCGAGCGGCG TCGGCTTGCT GGCTCGCGTC GCTCAGTCAC TCGCTCCTTC

3641  CGGAAGAGCG CCCAATACGC AAACCGCCTC TCCCCGCGCG TTGGCCGATT CATTAATGCA GCTGGCACGA
      GCCTTCTCGC GGGTTATGCG TTTGGCGGAG AGGGGCGCGC AACCGGCTAA GTAATTACGT CGACCGTGCT

3711  CAGGTTTCCC GACTGGAAAG CGGGCAGTGA GCGCAACGCA ATTAATGTGA GTTAGCTCAC TCATTAGGCA
      GTCCAAAGGG CTGACCTTTC GCCCGTCACT CGCGTTGCGT TAATTACACT CAATCGAGTG AGTAATCCGT

3781  CCCCAGGCTT TACACTTTAT GCTTCCGGCT CGTATGTTGT GTGGAATTGT GAGCGGATAA CAATTTCACA
      GGGGTCCGAA ATGTGAAATA CGAAGGCCGA GCATACAACA CACCTTAACA CTCGCCTATT GTTAAAGTGT
```

FIG._41F

```
                                       BssHII                               EcoRI
                                       ~~~~~~                                  ~~
3851  CAGGAAACAG CTATGACCAT GATTACGCCA AGCGCGCAAT TAACCCTCAC TAAAGGGAAC AAAAGCTGGA
      GTCCTTTGTC GATACTGGTA CTAATGCGGT TCGCGCGTTA ATTGGGAGTG ATTTCCCTTG TTTTCGACCT

      EcoRI
      ~~~~
3921  ATTCCACAAT GAACAATAAT AAGATTAAAA TAGCTTGCCC CCGTTGCAGC GATGGGTATT TTTTCTAGTA
      TAAGGTGTTA CTTGTTATTA TTCTAATTTT ATCGAACGGG GGCAACGTCG CTACCCATAA AAAAGATCAT

3991  AAATAAAAGA TAAACTTAGA CTCAAAACAT TTACAAAAAC AACCCCTAAA GTCCTAAAGC CCAAAGTGCT
      TTTATTTTCT ATTTGAATCT GAGTTTTGTA AATGTTTTTG TTGGGGATTT CAGGATTTCG GGTTTCACGA

4061  ATGCACGATC CATAGCAAGC CCAGCCCAAC CCAACCCAAC CCAACCCACC CCAGTGCAGC CAACTGGCAA
      TACGTGCTAG GTATCGTTCG GGTCGGGTTG GGTTGGGTTG GGTTGGGTGG GGTCACGTCG GTTGACCGTT

4131  ATAGTCTCCA CCCCCGGCAC TATCACCGTG AGTTGTCCGC ACCACCGCAC GTCTCGCAGC CAAAAAAAAA
      TATCAGAGGT GGGGGCCGTG ATAGTGGCAC TCAACAGGCG TGGTGGCGTG CAGAGCGTCG GTTTTTTTTT

4201  AAAAGAAAGA AAAAAAAGAA AAAGAAAAAC AGCAGGTGGG TCCGGGTCGT GGGGGCCGGA AAAGCGAGGA
      TTTTCTTTCT TTTTTTTCTT TTTCTTTTTG TCGTCCACCC AGGCCCAGCA CCCCCGGCCT TTTCGCTCCT

4271  GGATCGCGAG CAGCGACGAG GCCCGGCCCT CCCTCCGCTT CCAAAGAAAC GCCCCCCATC GCCACTATAT
      CCTAGCGCTC GTCGCTGCTC CGGGCCGGGA GGGAGGCGAA GGTTTCTTTG CGGGGGGTAG CGGTGATATA

4341  ACATACCCCC CCCTCTCCTC CCATCCCCCC AACCCTACCA CCACCACCAC CACCACCTCC TCCCCCCTCG
      TGTATGGGGG GGGAGAGGAG GGTAGGGGGG TTGGGATGGT GGTGGTGGTG GTGGTGGAGG AGGGGGGAGC

4411  CTGCCGGACG ACGAGCTCCT CCCCCCTCCC CCTCCGCCGC CGCCGGTAAC CACCCCGCCC CTCTCCTCTT
      GACGGCCTGC TGCTCGAGGA GGGGGGAGGG GGAGGCGGCG GCGGCCATTG GTGGGGCGGG GAGAGGAGAA

4481  TCTTTCTCCG TTTTTTTTTT CGTCTCGGTC TCGATCTTTG GCCTTGGTAG TTTGGGTGGG CGAGAGCGGC
      AGAAAGAGGC AAAAAAAAAA GCAGAGCCAG AGCTAGAAAC CGGAACCATC AAACCCACCC GCTCTCGCCG

4551  TTCGTCGCCC AGATCGGTGC GCGGGAGGGG CGGGATCTCG CGGCTGGCGT CTCCGGGCGT GAGTCGGCCC
      AAGCAGCGGG TCTAGCCACG CGCCCTCCCC GCCCTAGAGC GCCGACCGCA GAGGCCCGCA CTCAGCCGGG
```

FIG._41G

```
        BamHI                                            BglII
        ~~~~~~                                           ~~~~~~
4621    GGATCCTCGC GGGGAATGGG GCTCTCGGAT GTAGATCTTC TTTCTTTCTT CTTTTTGTGG TAGAATTTGA
        CCTAGGAGCG CCCCTTACCC CGAGAGCCTA CATCTAGAAG AAAGAAAGAA GAAAAACACC ATCTTAAACT

4691    ATCCCTCAGC ATTGTTCATC GGTAGTTTTT CTTTTCATGA TTTGTGACAA ATGCAGCCTC GTGCGGAGCT
        TAGGGAGTCG TAACAAGTAG CCATCAAAAA GAAAAGTACT AAACACTGTT TACGTCGGAG CACGCCTCGA

4761    TTTTTGTAGG TAG
        AAAAACATCC ATC
```

FIG._41H

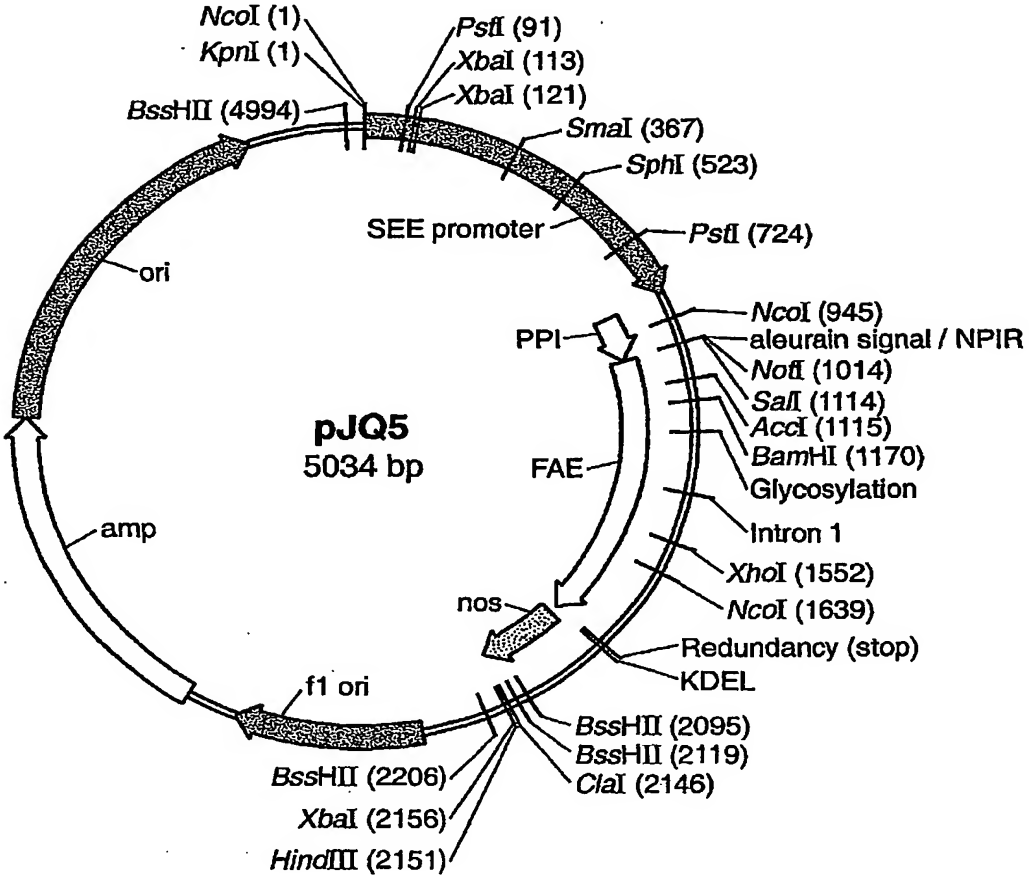

NcoI (1)
KpnI (1)
BssHII (4994)
PstI (91)
XbaI (113)
XbaI (121)
SmaI (367)
SphI (523)
SEE promoter
PstI (724)
ori
NcoI (945)
PPI
aleurain signal / NPIR
NotI (1014)
SalI (1114)
AccI (1115)
pJQ5
5034 bp
BamHI (1170)
FAE
Glycosylation
amp
Intron 1
XhoI (1552)
NcoI (1639)
nos
Redundancy (stop)
KDEL
f1 ori
BssHII (2095)
BssHII (2119)
ClaI (2146)
BssHII (2206)
XbaI (2156)
HindIII (2151)

FIG._42A

```
       NcoI
       ~~~~~~
       KpnI
       ~
   1   CATGGGCCAG GTATAATTAT GGGATATCTC AAGCAAATAA TCGAAATATC ACCATTGGCT ACAATATCTG
       GTACCCGGTC CATATTAATA CCCTATAGAG TTCGTTTATT AGCTTTATAG TGGTAACCGA TGTTATAGAC

                        PstI                                  XbaI       XbaI
                      ~~~~~~~~                              ~~~~~~     ~~~~~~~~
  71   AGCTCCGAGT TCTGACTGCA GTCTGGATGA CGCGTGTTGT ATCTAGAACT CTAGATAGCA CAGCCACAGC
       TCGAGGCTCA AGACTGACGT CAGACCTACT GCGCACAACA TAGATCTTGA GATCTATCGT GTCGGTGTCG

 141   ACCTACAGGA GTGCGACACT TGTGGACTGT AGTAGTGTTG GAGACGGAGC TCTTTCCTAC CTCCTGACGT
       TGGATGTCCT CACGCTGTGA ACACCTGACA TCATCACAAC CTCTGCCTCG AGAAAGGATG GAGGACTGCA

 211   TGCCGCCGTT GTCCATTCCA ACGGCATCAC TCTCAACCAA TCACGCGCTC CCAACAAAAT ATCGTCCCCC
       ACGGCGGCAA CAGGTAAGGT TGCCGTAGTG AGAGTTGGTT AGTGCGCGAG GGTTGTTTTA TAGCAGGGGG

 281   ATGTCTTGGC GGAGAGAGAG TACATACATG CTGTCGCGCC GTTTTTGTCT GAATCTCGCT TCCACTGGCC
       TACAGAACCG CCTCTCTCTC ATGTATGTAC GACAGCGCGG CAAAAACAGA CTTAGAGCGA AGGTGACCGG

                     SmaI
                    ~~~~~~
 351   AATCAGCTCA GCTCCCGGGA GCTCACTCAT TCAAGATCCC ATCGTCGTCG TCACCCCTGG CGTCATGGGA
       TTAGTCGAGT CGAGGGCCCT CGAGTGAGTA AGTTCTAGGG TAGCAGCAGC AGTGGGGACC GCAGTACCCT

 421   TGGAAAAGAA CCTCCGTTGC TCGGATGAGT CAGCCATATC CCCGAACAGA GTACTGCAAG ATAACCCAAT
       ACCTTTTCTT GGAGGCAACG AGCCTACTCA GTCGGTATAG GGGCTTGTCT CATGACGTTC TATTGGGTTA

                                    SphI
                                  ~~~~~~~
 491   TCAGATTCCC CCAATAGAGA AAGTATAGCA TGCTTTCGGG TTTTGTTTGG CTTAATTGAC TTTATTTTTG
       AGTCTAAGGG GGTTATCTCT TTCATATCGT ACGAAAGCCC AAAACAAACC GAATTAACTG AAATAAAAAC

 561   TTGGAGTTGA ATGCTGATTT GTTGTGTAAA ATGCCCAACC ATCTGAATAT CGAGACGGAT AATAGGCTGG
       AACCTCAACT TACGACTAAA CAACACATTT TACGGGTTGG TAGACTTATA GCTCTGCCTA TTATCCGACC
```

FIG._42B

```
631   CTAATTAATT TATAGCAAGA TTCTGTAGTG CACATCGCAA ATATCTTTCT GGGCATTACA GCTGGAGGCT
      GATTAATTAA ATATCGTTCT AAGACATCAC GTGTAGCGTT TATAGAAAGA CCCGTAATGT CGACCTCCGA

                           PstI
                         ~~~~~~~~
701   TCATCAGCCT GAAACACTCT GCAGAGCCTG AAGCAAGTGG TGAAGCGTGG CGATGAGATG GGTATAAAAC
      AGTAGTCGGA CTTTGTGAGA CGTCTCGGAC TTCGTTCACC ACTTCGCACC GCTACTCTAC CCATATTTTG

771   CCCCGGCACC GGGACGCGAG CTCCCGCCTA CCAGTACCAT CTCGCCTCGC TCCCCCTGCC GGACGACCCA
      GGGGCCGTGG CCCTGCGCTC GAGGGCGGAT GGTCATGGTA GAGCGGAGCG AGGGGGACGG CCTGCTGGGT

841   GTAAAATACT GTTGCCCACT CGCCGGCGAG ATGGMCGTGC ACAAGGAGGT SAACTTCGTS GCCTACCTCC
      CATTTTATGA CAACGGGTGA GCGGCCGCTC TACCKGCACG TGTTCCTCCA STTGAAGCAS CGGATGGAGG

                                           NcoI
                                          ~~~~~~
911   TGATCGTSCT CGGCCTCCTC TTGCTCGTST CCGCCATGGA GCACGTGGAC GCCAAGGCCT GCACCCKCGA
      ACTAGCASGA GCCGGAGGAG AACGAGCASA GGCGGTACCT CGTGCACCTG CGGTTCCGGA CGTGGGMGCT

                                          NotI
                                        ~~~~~~~~
981   GTGCGGCAAC CTCGGCTTCG GCATCTGCCC GGCGGCCGCC TCCACGCAGG GCATCTCCGA AGACCTCTAC
      CACGCCGTTG GAGCCGAAGC CGTAGACGGG CCGCCGGCGG AGGTGCGTCC CGTAGAGGCT TCTGGAGATG

                                                                           SalI
                                                                          ~~~~~~
                                                                           AccI
                                                                          ~~~~~~
1051  AGCCGTTTAG TCGAAATGGC CACTATCTCC CAAGCTGCCT ACGCCGACCT GTGCAACATT CCGTCGACTA
      TCGGCAAATC AGCTTTACCG GTGATAGAGG GTTCGACGGA TGCGGCTGGA CACGTTGTAA GGCAGCTGAT

                                                          BamHI
                                                         ~~~~~~~~
1121  TTATCAAGGG AGAGAAAATT TACAATTCTC AAACTGACAT TAACGGATGG ATCCTCCGCG ACGACAGCAG
      AATAGTTCCC TCTCTTTTAA ATGTTAAGAG TTTGACTGTA ATTGCCTACC TAGGAGGCGC TGCTGTCGTC
```

FIG._42C

```
1191  CAAAGAAATA ATCACCGTCT TCCGTGGCAC TGGTAGTGAT ACGAATCTAC AACTCGATAC TAACTACACC
      GTTTCTTTAT TAGTGGCAGA AGGCACCGTG ACCATCACTA TGCTTAGATG TTGAGCTATG ATTGATGTGG

1261  CTCACGCCTT TCGACACCCT ACCACAATGC AACGGTTGTG AAGTACACGG TGGATATTAT ATTGGATGGG
      GAGTGCGGAA AGCTGTGGGA TGGTGTTACG TTGCCAACAC TTCATGTGCC ACCTATAATA TAACCTACCC

1331  TCTCCGTCCA GGACCAAGTC GAGTCGCTTG TCAAACAGCA GGTTAGCCAG TATCCGGACT ACGCGCTGAC
      AGAGGCAGGT CCTGGTTCAG CTCAGCGAAC AGTTTGTCGT CCAATCGGTC ATAGGCCTGA TGCGCGACTG

1401  CGTGACCGGC CACKCCCTCG GCGCCTCCCT GGCGGCACTC ACTGCCGCCC AGCTGTCTGC GACATACGAC
      GCACTGGCCG GTGMGGGAGC CGCGGAGGGA CCGCCGTGAG TGACGGCGGG TCGACAGACG CTGTATGCTG

1471  AACATCCGCC TGTACACCTT CGGCGAACCG CGCAGCGGCA ATCAGGCCTT CGCGTCGTAC ATGAACGATG
      TTGTAGGCGG ACATGTGGAA GCCGCTTGGC GCGTCGCCGT TAGTCCGGAA GCGCAGCATG TACTTGCTAC

                 XhoI
                ~~~~~~
1541  CCTTCCAAGC CTCGAGCCCA GATACGACGC AGTATTTCCG GGTCACTCAT GCCAACGACG GCATCCCAAA
      GGAAGGTTCG GAGCTCGGGT CTATGCTGCG TCATAAAGGC CCAGTGAGTA CGGTTGCTGC CGTAGGGTTT

                                    NcoI
                                  ~~~~~~~
1611  CCTGCCCCCG GTGGAGCAGG GGTACGCCCA TGGCGGTGTA GAGTACTGGA GCGTTGATCC TTACAGCGCC
      GGACGGGGGC CACCTCGTCC CCATGCGGGT ACCGCCACAT CTCATGACCT CGCAACTAGG AATGTCGCGG

1681  CAGAACACAT TTGTCTGCAC TGGGGATGAA GTGCAGTGCT GTGAGGCCCA GGGCGGACAG GGTGTGAATA
      GTCTTGTGTA AACAGACGTG ACCCCTACTT CACGTCACGA CACTCCGGGT CCCGCCTGTC CCACACTTAT

1751  ATGCGCACAC GACTTATTTT GGGATGACGA GCGGAGCCTG TACATGGTGA TCAGTCATTT CAGCCTCCCC
      TACGCGTGTG CTGAATAAAA CCCTACTGCT CGCCTCGGAC ATGTACCACT AGTCAGTAAA GTCGGAGGGG

1821  GAGTGTACCA GGAAAGATGG ATGTCCTGGA GAGGGGGCCG CGTAACCACT GAAGGATGAG CTGTAAAGAA
      CTCACATGGT CCTTTCTACC TACAGGACCT CTCCCCCGGC GCATTGGTGA CTTCCTACTC GACATTTCTT
```

FIG._42D

```
1891  GCAGATCGTT CAAACATTTG GCAATAAAGT TTCTTAAGAT TGAATCCTGT TGCCGGTCTT GCGATGATTA
      CGTCTAGCAA GTTTGTAAAC CGTTATTTCA AAGAATTCTA ACTTAGGACA ACGGCCAGAA CGCTACTAAT

1961  TCATATAATT TCTGTTGAAT TACGTTAAGC ATGTAATAAT TAACATGTAA TGCATGACGT TATTTATGAG
      AGTATATTAA AGACAACTTA ATGCAATTCG TACATTATTA ATTGTACATT ACGTACTGCA ATAAATACTC

                                                                           BssHII
                                                                           ~~~~~~
2031  ATGGGTTTTT ATGATTAGAG TCCCGCAATT ATACATTTAA TACGCGATAG AAAACAAAAT ATAGCGCGCA
      TACCCAAAAA TACTAATCTC AGGGCGTTAA TATGTAAATT ATGCGCTATC TTTTGTTTTA TATCGCGCGT

                                                                  XbaI
                                                                 ~~~~~~
                        BssHII                            ClaI HindIII
                       ~~~~~~~                           ~~~~~~~~~~~~~
2101  AACTAGGATA AATTATCGCG CGCGGTGTCA TCTATGTTAC TAGATCGATA AGCTTCTAGA GCGGCCGGTG
      TTGATCCTAT TTAATAGCGC GCGCCACAGT AGATACAATG ATCTAGCTAT TCGAAGATCT CGCCGGCCAC

                                           BssHII
                                           ~~~~~~
2171  GAGCTCCAAT TCGCCCTATA GTGAGTCGTA TTACGCGCGC TCACTGGCCG TCGTTTTACA ACGTCGTGAC
      CTCGAGGTTA AGCGGGATAT CACTCAGCAT AATGCGCGCG AGTGACCGGC AGCAAAATGT TGCAGCACTG

2241  TGGGAAAACC CTGGCGTTAC CCAACTTAAT CGCCTTGCAG CACATCCCCC TTTCGCCAGC TGGCGTAATA
      ACCCTTTTGG GACCGCAATG GGTTGAATTA GCGGAACGTC GTGTAGGGGG AAAGCGGTCG ACCGCATTAT

2311  GCGAAGAGGC CCGCACCGAT CGCCCTTCCC AACAGTTGCG CAGCCTGAAT GGCGAATGGG ACGCGCCCTG
      CGCTTCTCCG GGCGTGGCTA GCGGGAAGGG TTGTCAACGC GTCGGACTTA CCGCTTACCC TGCGCGGGAC

2381  TAGCGGCGCA TTAAGCGCGG CGGGTGTGGT GGTTACGCGC AGCGTGACCG CTACACTTGC CAGCGCCCTA
      ATCGCCGCGT AATTCGCGCC GCCCACACCA CCAATGCGCG TCGCACTGGC GATGTGAACG GTCGCGGGAT

2451  GCGCCCGCTC CTTTCGCTTT CTTCCCTTCC TTTCTCGCCA CGTTCGCCGG CTTTCCCCGT CAAGCTCTAA
      CGCGGGCGAG GAAAGCGAAA GAAGGGAAGG AAAGAGCGGT GCAAGCGGCC GAAAGGGGCA GTTCGAGATT
```

FIG._42E

```
2521  ATCGGGGGCT CCCTTTAGGG TTCCGATTTA GTGCTTTACG GCACCTCGAC CCCAAAAAAC TTGATTAGGG
      TAGCCCCCGA GGGAAATCCC AAGGCTAAAT CACGAAATGC CGTGGAGCTG GGGTTTTTTG AACTAATCCC

2591  TGATGGTTCA CGTAGTGGGC CATCGCCCTG ATAGACGGTT TTTCGCCCTT TGACGTTGGA GTCCACGTTC
      ACTACCAAGT GCATCACCCG GTAGCGGGAC TATCTGCCAA AAAGCGGGAA ACTGCAACCT CAGGTGCAAG

2661  TTTAATAGTG GACTCTTGTT CCAAACTGGA ACAACACTCA ACCCTATCTC GGTCTATTCT TTTGATTTAT
      AAATTATCAC CTGAGAACAA GGTTTGACCT TGTTGTGAGT TGGGATAGAG CCAGATAAGA AAACTAAATA

2731  AAGGGATTTT GCCGATTTCG GCCTATTGGT TAAAAAATGA GCTGATTTAA CAAAAATTTA ACGCGAATTT
      TTCCCTAAAA CGGCTAAAGC CGGATAACCA ATTTTTTACT CGACTAAATT GTTTTTAAAT TGCGCTTAAA

2801  TAACAAAATA TTAACGCTTA CAATTTAGGT GGCACTTTTC GGGGAAATGT GCGCGGAACC CCTATTTGTT
      ATTGTTTTAT AATTGCGAAT GTTAAATCCA CCGTGAAAAG CCCCTTTACA CGCGCCTTGG GGATAAACAA

2871  TATTTTTCTA AATACATTCA AATATGTATC CGCTCATGAG ACAATAACCC TGATAAATGC TTCAATAATA
      ATAAAAAGAT TTATGTAAGT TTATACATAG GCGAGTACTC TGTTATTGGG ACTATTTACG AAGTTATTAT

2941  TTGAAAAAGG AAGAGTATGA GTATTCAACA TTTCCGTGTC GCCCTTATTC CCTTTTTTGC GGCATTTTGC
      AACTTTTTCC TTCTCATACT CATAAGTTGT AAAGGCACAG CGGGAATAAG GGAAAAAACG CCGTAAAACG

3011  CTTCCTGTTT TTGCTCACCC AGAAACGCTG GTGAAAGTAA AAGATGCTGA AGATCAGTTG GGTGCACGAG
      GAAGGACAAA AACGAGTGGG TCTTTGCGAC CACTTTCATT TTCTACGACT TCTAGTCAAC CCACGTGCTC

3081  TGGGTTACAT CGAACTGGAT CTCAACAGCG GTAAGATCCT TGAGAGTTTT CGCCCCGAAG AACGTTTTCC
      ACCCAATGTA GCTTGACCTA GAGTTGTCGC CATTCTAGGA ACTCTCAAAA GCGGGGCTTC TTGCAAAAGG

3151  AATGATGAGC ACTTTTAAAG TTCTGCTATG TGGCGCGGTA TTATCCCGTA TTGACGCCGG GCAAGAGCAA
      TTACTACTCG TGAAAATTTC AAGACGATAC ACCGCGCCAT AATAGGGCAT AACTGCGGCC CGTTCTCGTT

3221  CTCGGTCGCC GCATACACTA TTCTCAGAAT GACTTGGTTG AGTACTCACC AGTCACAGAA AAGCATCTTA
      GAGCCAGCGG CGTATGTGAT AAGAGTCTTA CTGAACCAAC TCATGAGTGG TCAGTGTCTT TTCGTAGAAT

3291  CGGATGGCAT GACAGTAAGA GAATTATGCA GTGCTGCCAT AACCATGAGT GATAACACTG CGGCCAACTT
      GCCTACCGTA CTGTCATTCT CTTAATACGT CACGACGGTA TTGGTACTCA CTATTGTGAC GCCGGTTGAA
```

FIG._42F

```
3361  ACTTCTGACA ACGATCGGAG GACCGAAGGA GCTAACCGCT TTTTTGCACA ACATGGGGGA TCATGTAACT
      TGAAGACTGT TGCTAGCCTC CTGGCTTCCT CGATTGGCGA AAAAACGTGT TGTACCCCCT AGTACATTGA

3431  CGCCTTGATC GTTGGGAACC GGAGCTGAAT GAAGCCATAC CAAACGACGA GCGTGACACC ACGATGCCTG
      GCGGAACTAG CAACCCTTGG CCTCGACTTA CTTCGGTATG GTTTGCTGCT CGCACTGTGG TGCTACGGAC

3501  TAGCAATGGC AACAACGTTG CGCAAACTAT TAACTGGCGA ACTACTTACT CTAGCTTCCC GGCAACAATT
      ATCGTTACCG TTGTTGCAAC GCGTTTGATA ATTGACCGCT TGATGAATGA GATCGAAGGG CCGTTGTTAA

3571  AATAGACTGG ATGGAGGCGG ATAAAGTTGC AGGACCACTT CTGCGCTCGG CCCTTCCGGC TGGCTGGTTT
      TTATCTGACC TACCTCCGCC TATTTCAACG TCCTGGTGAA GACGCGAGCC GGGAAGGCCG ACCGACCAAA

3641  ATTGCTGATA AATCTGGAGC CGGTGAGCGT GGGTCTCGCG GTATCATTGC AGCACTGGGG CCAGATGGTA
      TAACGACTAT TTAGACCTCG GCCACTCGCA CCCAGAGCGC CATAGTAACG TCGTGACCCC GGTCTACCAT

3711  AGCCCTCCCG TATCGTAGTT ATCTACACGA CGGGGAGTCA GGCAACTATG GATGAACGAA ATAGACAGAT
      TCGGGAGGGC ATAGCATCAA TAGATGTGCT GCCCCTCAGT CCGTTGATAC CTACTTGCTT TATCTGTCTA

3781  CGCTGAGATA GGTGCCTCAC TGATTAAGCA TTGGTAACTG TCAGACCAAG TTTACTCATA TATACTTTAG
      GCGACTCTAT CCACGGAGTG ACTAATTCGT AACCATTGAC AGTCTGGTTC AAATGAGTAT ATATGAAATC

3851  ATTGATTTAA AACTTCATTT TTAATTTAAA AGGATCTAGG TGAAGATCCT TTTTGATAAT CTCATGACCA
      TAACTAAATT TTGAAGTAAA AATTAAATTT TCCTAGATCC ACTTCTAGGA AAAACTATTA GAGTACTGGT

3921  AAATCCCTTA ACGTGAGTTT TCGTTCCACT GAGCGTCAGA CCCCGTAGAA AAGATCAAAG GATCTTCTTG
      TTTAGGGAAT TGCACTCAAA AGCAAGGTGA CTCGCAGTCT GGGGCATCTT TTCTAGTTTC CTAGAAGAAC

3991  AGATCCTTTT TTTCTGCGCG TAATCTGCTG CTTGCAAACA AAAAAACCAC CGCTACCAGC GGTGGTTTGT
      TCTAGGAAAA AAAGACGCGC ATTAGACGAC GAACGTTTGT TTTTTTGGTG GCGATGGTCG CCACCAAACA

4061  TTGCCGGATC AAGAGCTACC AACTCTTTTT CCGAAGGTAA CTGGCTTCAG CAGAGCGCAG ATACCAAATA
      AACGGCCTAG TTCTCGATGG TTGAGAAAAA GGCTTCCATT GACCGAAGTC GTCTCGCGTC TATGGTTTAT

4131  CTGTCCTTCT AGTGTAGCCG TAGTTAGGCC ACCACTTCAA GAACTCTGTA GCACCGCCTA CATACCTCGC
      GACAGGAAGA TCACATCGGC ATCAATCCGG TGGTGAAGTT CTTGAGACAT CGTGGCGGAT GTATGGAGCG
```

FIG._42G

```
4201  TCTGCTAATC CTGTTACCAG TGGCTGCTGC CAGTGGCGAT AAGTCGTGTC TTACCGGGTT GGACTCAAGA
      AGACGATTAG GACAATGGTC ACCGACGACG GTCACCGCTA TTCAGCACAG AATGGCCCAA CCTGAGTTCT

4271  CGATAGTTAC CGGATAAGGC GCAGCGGTCG GGCTGAACGG GGGGTTCGTG CACACAGCCC AGCTTGGAGC
      GCTATCAATG GCCTATTCCG CGTCGCCAGC CCGACTTGCC CCCCAAGCAC GTGTGTCGGG TCGAACCTCG

4341  GAACGACCTA CACCGAACTG AGATACCTAC AGCGTGAGCT ATGAGAAAGC GCCACGCTTC CCGAAGGGAG
      CTTGCTGGAT GTGGCTTGAC TCTATGGATG TCGCACTCGA TACTCTTTCG CGGTGCGAAG GGCTTCCCTC

4411  AAAGGCGGAC AGGTATCCGG TAAGCGGCAG GGTCGGAACA GGAGAGCGCA CGAGGGAGCT TCCAGGGGGA
      TTTCCGCCTG TCCATAGGCC ATTCGCCGTC CCAGCCTTGT CCTCTCGCGT GCTCCCTCGA AGGTCCCCCT

4481  AACGCCTGGT ATCTTTATAG TCCTGTCGGG TTTCGCCACC TCTGACTTGA GCGTCGATTT TTGTGATGCT
      TTGCGGACCA TAGAAATATC AGGACAGCCC AAAGCGGTGG AGACTGAACT CGCAGCTAAA AACACTACGA

4551  CGTCAGGGGG GCGGAGCCTA TGGAAAAACG CCAGCAACGC GGCCTTTTTA CGGTTCCTGG CCTTTTGCTG
      GCAGTCCCCC CGCCTCGGAT ACCTTTTTGC GGTCGTTGCG CCGGAAAAAT GCCAAGGACC GGAAAACGAC

4621  GCCTTTTGCT CACATGTTCT TTCCTGCGTT ATCCCCTGAT TCTGTGGATA ACCGTATTAC CGCCTTTGAG
      CGGAAAACGA GTGTACAAGA AAGGACGCAA TAGGGGACTA AGACACCTAT TGGCATAATG GCGGAAACTC

4691  TGAGCTGATA CCGCTCGCCG CAGCCGAACG ACCGAGCGCA GCGAGTCAGT GAGCGAGGAA GCGGAAGAGC
      ACTCGACTAT GGCGAGCGGC GTCGGCTTGC TGGCTCGCGT CGCTCAGTCA CTCGCTCCTT CGCCTTCTCG

4761  GCCCAATACG CAAACCGCCT CTCCCCGCGC GTTGGCCGAT TCATTAATGC AGCTGGCACG ACAGGTTTCC
      CGGGTTATGC GTTTGGCGGA GAGGGGCGCG CAACCGGCTA AGTAATTACG TCGACCGTGC TGTCCAAAGG

4831  CGACTGGAAA GCGGGCAGTG AGCGCAACGC AATTAATGTG AGTTAGCTCA CTCATTAGGC ACCCCAGGCT
      GCTGACCTTT CGCCCGTCAC TCGCGTTGCG TTAATTACAC TCAATCGAGT GAGTAATCCG TGGGGTCCGA

4901  TTACACTTTA TGCTTCCGGC TCGTATGTTG TGTGGAATTG TGAGCGGATA ACAATTTCAC ACAGGAAACA
      AATGTGAAAT ACGAAGGCCG AGCATACAAC ACACCTTAAC ACTCGCCTAT TGTTAAAGTG TGTCCTTTGT
```

FIG._42H

```
                                                                        NcoI
                              BssHII                                    KpnI
                              ~~~~~~                                  ~~~~~
4971  GCTATGACCA TGATTACGCC AAGCGCGCAA TTAACCCTCA CTAAAGGGAA CAAAAGCTGG GTAC
      CGATACTGGT ACTAATGCGG TTCGCGCGTT AATTGGGAGT GATTTCCCTT GTTTTCGACC CATG
```

FIG._42I

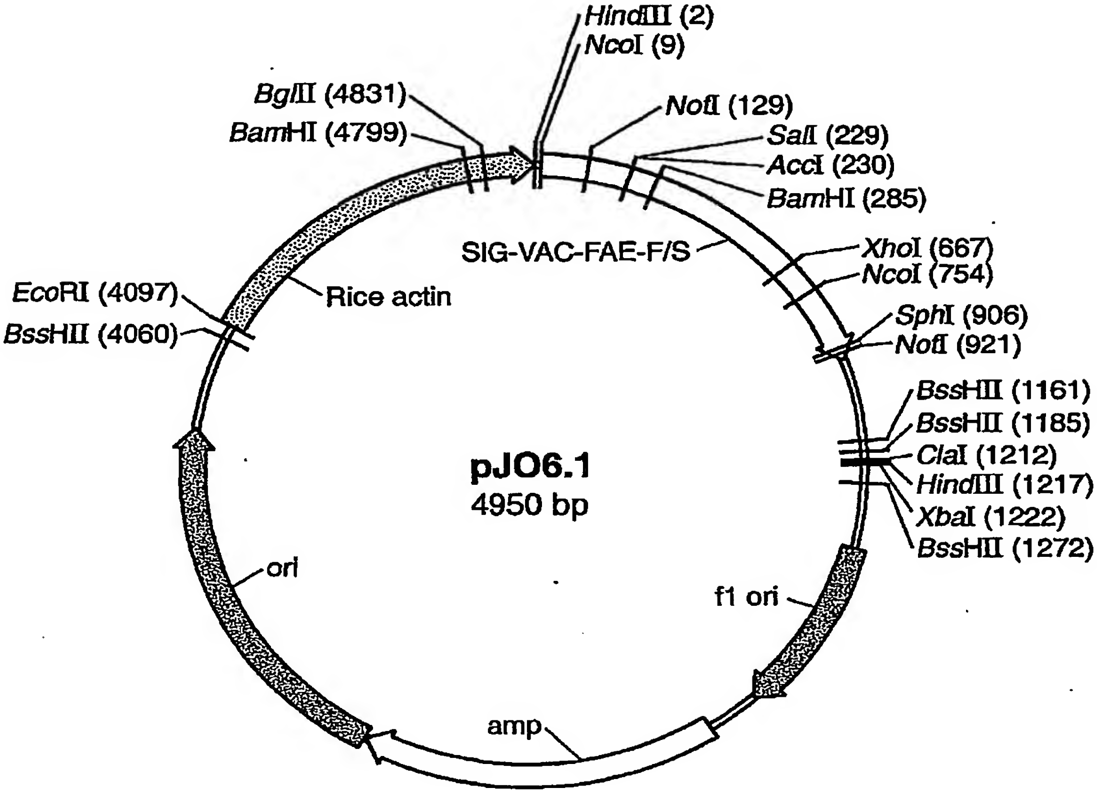

HindIII (2)
NcoI (9)
NotI (129)
SalI (229)
AccI (230)
BamHI (285)
XhoI (667)
NcoI (754)
SphI (906)
NotI (921)
BssHII (1161)
BssHII (1185)
ClaI (1212)
HindIII (1217)
XbaI (1222)
BssHII (1272)
BglII (4831)
BamHI (4799)
EcoRI (4097)
BssHII (4060)
SIG-VAC-FAE-F/S
Rice actin
pJO6.1
4950 bp
ori
f1 ori
amp

FIG._43A

```
     HindIII NcoI
     ~~~~~~ ~~~~~~~
  1  AAGCTTACCA TGGCCCACGC CCGCGTCCTC CTCCTGGCGC TCGCCGTGCT GGCCACGGCC GCCGTCGCCG
     TTCGAATGGT ACCGGGTGCG GGCGCAGGAG GAGGACCGCG AGCGGCACGA CCGGTGCCGG CGGCAGCGGC

                                                                  NotI
                                                                ~~~~~~~~~
 71  TCGCCTCCTC CTCCTCCTTC GCCGACTCCA ACCCGATCCG GCCCGTCACC GACCGCGCGG CCGCCTCCAC
     AGCGGAGGAG GAGGAGGAAG CGGCTGAGGT TGGGCTAGGC CGGGCAGTGG CTGGCGCGCC GGCGGAGGTG

141  GCAGGGCATC TCCGAAGACC TCTACAGCCG TTTAGTCGAA ATGGCCACTA TCTCCCAAGC TGCCTACGCC
     CGTCCCGTAG AGGCTTCTGG AGATGTCGGC AAATCAGCTT TACCGGTGAT AGAGGGTTCG ACGGATGCGG

                          SalI
                         ~~~~~~~
                          AccI
                         ~~~~~~~
211  GACCTGTGCA ACATTCCGTC GACTATTATC AAGGGAGAGA AAATTTACAA TTCTCAAACT GACATTAACG
     CTGGACACGT TGTAAGGCAG CTGATAATAG TTCCCTCTCT TTTAAATGTT AAGAGTTTGA CTGTAATTGC

        BamHI
       ~~~~~~
281  GATGGATCCT CCGCGACGAC AGCAGCAAAG AAATAATCAC CGTCTTCCGT GGCACTGGTA GTGATACGAA
     CTACCTAGGA GGCGCTGCTG TCGTCGTTTC TTTATTAGTG GCAGAAGGCA CCGTGACCAT CACTATGCTT

351  TCTACAACTC GATACTAACT ACACCCTCAC GCCTTTCGAC ACCCTACCAC AATGCAACGG TTGTGAAGTA
     AGATGTTGAG CTATGATTGA TGTGGGAGTG CGGAAAGCTG TGGGATGGTG TTACGTTGCC AACACTTCAT

421  CACGGTGGAT ATTATATTGG ATGGGTCTCC GTCCAGGACC AAGTCGAGTC GCTTGTCAAA CAGCAGGTTA
     GTGCCACCTA TAATATAACC TACCCAGAGG CAGGTCCTGG TTCAGCTCAG CGAACAGTTT GTCGTCCAAT

491  GCCAGTATCC GGACTACGCG CTGACCGTGA CCGGCCACKC CCTCGGCGCC TCCCTGGCGG CACTCACTGC
     CGGTCATAGG CCTGATGCGC GACTGGCACT GGCCGGTGMG GGAGCCGCGG AGGGACCGCC GTGAGTGACG

561  CGCCCAGCTG TCTGCGACAT ACGACAACAT CCGCCTGTAC ACCTTCGGCG AACCGCGCAG CGGCAATCAG
     GCGGGTCGAC AGACGCTGTA TGCTGTTGTA GGCGGACATG TGGAAGCCGC TTGGCGCGTC GCCGTTAGTC
```

FIG._43B

```
                                             XhoI
                                            ~~~~~~~~
631   GCCTTCGCGT CGTACATGAA CGATGCCTTC CAAGCCTCGA GCCCAGATAC GACGCAGTAT TTCCGGGTCA
      CGGAAGCGCA GCATGTACTT GCTACGGAAG GTTCGGAGCT CGGGTCTATG CTGCGTCATA AAGGCCCAGT

                                                               NcoI
                                                              ~~~~~~~
701   CTCATGCCAA CGACGGCATC CCAAACCTGC CCCCGGTGGA GCAGGGGTAC GCCCATGGCG GTGTAGAGTA
      GAGTACGGTT GCTGCCGTAG GGTTTGGACG GGGGCCACCT CGTCCCCATG CGGGTACCGC CACATCTCAT

771   CTGGAGCGTT GATCCTTACA GCGCCCAGAA CACATTTGTC TGCACTGGGG ATGAAGTGCA GTGCTGTGAG
      GACCTCGCAA CTAGGAATGT CGCGGGTCTT GTGTAAACAG ACGTGACCCC TACTTCACGT CACGACACTC

                                                                        SphI
                                                                        ~~~~~~~
841   GCCCAGGGCG GACAGGGTGT GAATAATGCG CACACGACTT ATTTTGGGAT GACGAGCGGC GCATGCACCT
      CGGGTCCCGC CTGTCCCACA CTTATTACGC GTGTGCTGAA TAAAACCCTA CTGCTCGCCG CGTACGTGGA

                NotI
             ~~~~~~~~~~
911   GGCCGGTCGC GGCCGCGGAA ACCACTGAAG GATGAGCTGT AAAGAAGCAG ATCGTTCAAA CATTTGGCAA
      CCGGCCAGCG CCGGCGCCTT TGGTGACTTC CTACTCGACA TTTCTTCGTC TAGCAAGTTT GTAAACCGTT

981   TAAAGTTTCT TAAGATTGAA TCCTGTTGCC GGTCTTGCGA TGATTATCAT ATAATTTCTG TTGAATTACG
      ATTTCAAAGA ATTCTAACTT AGGACAACGG CCAGAACGCT ACTAATAGTA TATTAAAGAC AACTTAATGC

1051  TTAAGCATGT AATAATTAAC ATGTAATGCA TGACGTTATT TATGAGATGG GTTTTTATGA TTAGAGTCCC
      AATTCGTACA TTATTAATTG TACATTACGT ACTGCAATAA ATACTCTACC CAAAAATACT AATCTCAGGG

                                                 BssHII                    BssHII
                                                ~~~~~~~~                   ~~~~~~~
1121  GCAATTATAC ATTTAATACG CGATAGAAAA CAAAATATAG CGCGCAAACT AGGATAAATT ATCGCGCGCG
      CGTTAATATG TAAATTATGC GCTATCTTTT GTTTTATATC GCGCGTTTGA TCCTATTTAA TAGCGCGCGC
```

FIG._43C

```
                                        XbaI
                                       ~~~~~~
                            ClaI HindIII
                          ~~~~~~~~~~~~~~
1191  GTGTCATCTA TGTTACTAGA TCGATAAGCT TCTAGAGCGG CCGGTGGAGC TCCAATTCGC CCTATAGTGA
      CACAGTAGAT ACAATGATCT AGCTATTCGA AGATCTCGCC GGCCACCTCG AGGTTAAGCG GGATATCACT

                 BssHII
                 ~~~~~~
1261  GTCGTATTAC GCGCGCTCAC TGGCCGTCGT TTTACAACGT CGTGACTGGG AAAACCCTGG CGTTACCCAA
      CAGCATAATG CGCGCGAGTG ACCGGCAGCA AAATGTTGCA GCACTGACCC TTTTGGGACC GCAATGGGTT

1331  CTTAATCGCC TTGCAGCACA TCCCCCTTTC GCCAGCTGGC GTAATAGCGA AGAGGCCCGC ACCGATCGCC
      GAATTAGCGG AACGTCGTGT AGGGGGAAAG CGGTCGACCG CATTATCGCT TCTCCGGGCG TGGCTAGCGG

1401  CTTCCCAACA GTTGCGCAGC CTGAATGGCG AATGGGACGC GCCCTGTAGC GGCGCATTAA GCGCGGCGGG
      GAAGGGTTGT CAACGCGTCG GACTTACCGC TTACCCTGCG CGGGACATCG CCGCGTAATT CGCGCCGCCC

1471  TGTGGTGGTT ACGCGCAGCG TGACCGCTAC ACTTGCCAGC GCCCTAGCGC CCGCTCCTTT CGCTTTCTTC
      ACACCACCAA TGCGCGTCGC ACTGGCGATG TGAACGGTCG CGGGATCGCG GGCGAGGAAA GCGAAAGAAG

1541  CCTTCCTTTC TCGCCACGTT CGCCGGCTTT CCCCGTCAAG CTCTAAATCG GGGGCTCCCT TTAGGGTTCC
      GGAAGGAAAG AGCGGTGCAA GCGGCCGAAA GGGGCAGTTC GAGATTTAGC CCCCGAGGGA AATCCCAAGG

1611  GATTTAGTGC TTTACGGCAC CTCGACCCCA AAAAACTTGA TTAGGGTGAT GGTTCACGTA GTGGGCCATC
      CTAAATCACG AAATGCCGTG GAGCTGGGGT TTTTTGAACT AATCCCACTA CCAAGTGCAT CACCCGGTAG

1681  GCCCTGATAG ACGGTTTTTC GCCCTTTGAC GTTGGAGTCC ACGTTCTTTA ATAGTGGACT CTTGTTCCAA
      CGGGACTATC TGCCAAAAAG CGGGAAACTG CAACCTCAGG TGCAAGAAAT TATCACCTGA GAACAAGGTT

1751  ACTGGAACAA CACTCAACCC TATCTCGGTC TATTCTTTTG ATTTATAAGG GATTTTGCCG ATTTCGGCCT
      TGACCTTGTT GTGAGTTGGG ATAGAGCCAG ATAAGAAAAC TAAATATTCC CTAAAACGGC TAAAGCCGGA

1821  ATTGGTTAAA AAATGAGCTG ATTTAACAAA AATTTAACGC GAATTTTAAC AAAATATTAA CGCTTACAAT
      TAACCAATTT TTTACTCGAC TAAATTGTTT TTAAATTGCG CTTAAAATTG TTTTATAATT GCGAATGTTA
```

FIG._43D

```
1891  TTAGGTGGCA CTTTTCGGGG AAATGTGCGC GGAACCCCTA TTTGTTTATT TTTCTAAATA CATTCAAATA
      AATCCACCGT GAAAAGCCCC TTTACACGCG CCTTGGGGAT AAACAAATAA AAAGATTTAT GTAAGTTTAT

1961  TGTATCCGCT CATGAGACAA TAACCCTGAT AAATGCTTCA ATAATATTGA AAAAGGAAGA GTATGAGTAT
      ACATAGGCGA GTACTCTGTT ATTGGGACTA TTTACGAAGT TATTATAACT TTTTCCTTCT CATACTCATA

2031  TCAACATTTC CGTGTCGCCC TTATTCCCTT TTTTGCGGCA TTTTGCCTTC CTGTTTTTGC TCACCCAGAA
      AGTTGTAAAG GCACAGCGGG AATAAGGGAA AAAACGCCGT AAAACGGAAG GACAAAAACG AGTGGGTCTT

2101  ACGCTGGTGA AAGTAAAAGA TGCTGAAGAT CAGTTGGGTG CACGAGTGGG TTACATCGAA CTGGATCTCA
      TGCGACCACT TTCATTTTCT ACGACTTCTA GTCAACCCAC GTGCTCACCC AATGTAGCTT GACCTAGAGT

2171  ACAGCGGTAA GATCCTTGAG AGTTTTCGCC CCGAAGAACG TTTTCCAATG ATGAGCACTT TTAAAGTTCT
      TGTCGCCATT CTAGGAACTC TCAAAAGCGG GGCTTCTTGC AAAAGGTTAC TACTCGTGAA AATTTCAAGA

2241  GCTATGTGGC GCGGTATTAT CCCGTATTGA CGCCGGGCAA GAGCAACTCG GTCGCCGCAT ACACTATTCT
      CGATACACCG CGCCATAATA GGGCATAACT GCGGCCCGTT CTCGTTGAGC CAGCGGCGTA TGTGATAAGA

2311  CAGAATGACT TGGTTGAGTA CTCACCAGTC ACAGAAAAGC ATCTTACGGA TGGCATGACA GTAAGAGAAT
      GTCTTACTGA ACCAACTCAT GAGTGGTCAG TGTCTTTTCG TAGAATGCCT ACCGTACTGT CATTCTCTTA

2381  TATGCAGTGC TGCCATAACC ATGAGTGATA ACACTGCGGC CAACTTACTT CTGACAACGA TCGGAGGACC
      ATACGTCACG ACGGTATTGG TACTCACTAT TGTGACGCCG GTTGAATGAA GACTGTTGCT AGCCTCCTGG

2451  GAAGGAGCTA ACCGCTTTTT TGCACAACAT GGGGGATCAT GTAACTCGCC TTGATCGTTG GGAACCGGAG
      CTTCCTCGAT TGGCGAAAAA ACGTGTTGTA CCCCCTAGTA CATTGAGCGG AACTAGCAAC CCTTGGCCTC

2521  CTGAATGAAG CCATACCAAA CGACGAGCGT GACACCACGA TGCCTGTAGC AATGGCAACA ACGTTGCGCA
      GACTTACTTC GGTATGGTTT GCTGCTCGCA CTGTGGTGCT ACGGACATCG TTACCGTTGT TGCAACGCGT

2591  AACTATTAAC TGGCGAACTA CTTACTCTAG CTTCCCGGCA ACAATTAATA GACTGGATGG AGGCGGATAA
      TTGATAATTG ACCGCTTGAT GAATGAGATC GAAGGGCCGT TGTTAATTAT CTGACCTACC TCCGCCTATT

2661  AGTTGCAGGA CCACTTCTGC GCTCGGCCCT TCCGGCTGGC TGGTTTATTG CTGATAAATC TGGAGCCGGT
      TCAACGTCCT GGTGAAGACG CGAGCCGGGA AGGCCGACCG ACCAAATAAC GACTATTTAG ACCTCGGCCA
```

FIG._43E

```
2731  GAGCGTGGGT CTCGCGGTAT CATTGCAGCA CTGGGGCCAG ATGGTAAGCC CTCCCGTATC GTAGTTATCT
      CTCGCACCCA GAGCGCCATA GTAACGTCGT GACCCCGGTC TACCATTCGG GAGGGCATAG CATCAATAGA

2801  ACACGACGGG GAGTCAGGCA ACTATGGATG AACGAAATAG ACAGATCGCT GAGATAGGTG CCTCACTGAT
      TGTGCTGCCC CTCAGTCCGT TGATACCTAC TTGCTTTATC TGTCTAGCGA CTCTATCCAC GGAGTGACTA

2871  TAAGCATTGG TAACTGTCAG ACCAAGTTTA CTCATATATA CTTTAGATTG ATTTAAAACT TCATTTTTAA
      ATTCGTAACC ATTGACAGTC TGGTTCAAAT GAGTATATAT GAAATCTAAC TAAATTTTGA AGTAAAAATT

2941  TTTAAAAGGA TCTAGGTGAA GATCCTTTTT GATAATCTCA TGACCAAAAT CCCTTAACGT GAGTTTTCGT
      AAATTTTCCT AGATCCACTT CTAGGAAAAA CTATTAGAGT ACTGGTTTTA GGGAATTGCA CTCAAAAGCA

3011  TCCACTGAGC GTCAGACCCC GTAGAAAAGA TCAAAGGATC TTCTTGAGAT CCTTTTTTTC TGCGCGTAAT
      AGGTGACTCG CAGTCTGGGG CATCTTTTCT AGTTTCCTAG AAGAACTCTA GGAAAAAAAG ACGCGCATTA

3081  CTGCTGCTTG CAAACAAAAA AACCACCGCT ACCAGCGGTG GTTTGTTTGC CGGATCAAGA GCTACCAACT
      GACGACGAAC GTTTGTTTTT TTGGTGGCGA TGGTCGCCAC CAAACAAACG GCCTAGTTCT CGATGGTTGA

3151  CTTTTTCCGA AGGTAACTGG CTTCAGCAGA GCGCAGATAC CAAATACTGT CCTTCTAGTG TAGCCGTAGT
      GAAAAAGGCT TCCATTGACC GAAGTCGTCT CGCGTCTATG GTTTATGACA GGAAGATCAC ATCGGCATCA

3221  TAGGCCACCA CTTCAAGAAC TCTGTAGCAC CGCCTACATA CCTCGCTCTG CTAATCCTGT TACCAGTGGC
      ATCCGGTGGT GAAGTTCTTG AGACATCGTG GCGGATGTAT GGAGCGAGAC GATTAGGACA ATGGTCACCG

3291  TGCTGCCAGT GGCGATAAGT CGTGTCTTAC CGGGTTGGAC TCAAGACGAT AGTTACCGGA TAAGGCGCAG
      ACGACGGTCA CCGCTATTCA GCACAGAATG GCCCAACCTG AGTTCTGCTA TCAATGGCCT ATTCCGCGTC

3361  CGGTCGGGCT GAACGGGGGG TTCGTGCACA CAGCCCAGCT TGGAGCGAAC GACCTACACC GAACTGAGAT
      GCCAGCCCGA CTTGCCCCCC AAGCACGTGT GTCGGGTCGA ACCTCGCTTG CTGGATGTGG CTTGACTCTA

3431  ACCTACAGCG TGAGCTATGA GAAAGCGCCA CGCTTCCCGA AGGGAGAAAG GCGGACAGGT ATCCGGTAAG
      TGGATGTCGC ACTCGATACT CTTTCGCGGT GCGAAGGGCT TCCCTCTTTC CGCCTGTCCA TAGGCCATTC

3501  CGGCAGGGTC GGAACAGGAG AGCGCACGAG GGAGCTTCCA GGGGGAAACG CCTGGTATCT TTATAGTCCT
      GCCGTCCCAG CCTTGTCCTC TCGCGTGCTC CCTCGAAGGT CCCCCTTTGC GGACCATAGA AATATCAGGA
```

FIG._43F

```
3571  GTCGGGTTTC GCCACCTCTG ACTTGAGCGT CGATTTTTGT GATGCTCGTC AGGGGGGCGG AGCCTATGGA
      CAGCCCAAAG CGGTGGAGAC TGAACTCGCA GCTAAAAACA CTACGAGCAG TCCCCCCGCC TCGGATACCT

3641  AAAACGCCAG CAACGCGGCC TTTTTACGGT TCCTGGCCTT TTGCTGGCCT TTTGCTCACA TGTTCTTTCC
      TTTTGCGGTC GTTGCGCCGG AAAAATGCCA AGGACCGGAA AACGACCGGA AAACGAGTGT ACAAGAAAGG

3711  TGCGTTATCC CCTGATTCTG TGGATAACCG TATTACCGCC TTTGAGTGAG CTGATACCGC TCGCCGCAGC
      ACGCAATAGG GGACTAAGAC ACCTATTGGC ATAATGGCGG AAACTCACTC GACTATGGCG AGCGGCGTCG

3781  CGAACGACCG AGCGCAGCGA GTCAGTGAGC GAGGAAGCGG AAGAGCGCCC AATACGCAAA CCGCCTCTCC
      GCTTGCTGGC TCGCGTCGCT CAGTCACTCG CTCCTTCGCC TTCTCGCGGG TTATGCGTTT GGCGGAGAGG

3851  CCGCGCGTTG GCCGATTCAT TAATGCAGCT GGCACGACAG GTTTCCCGAC TGGAAAGCGG GCAGTGAGCG
      GGCGCGCAAC CGGCTAAGTA ATTACGTCGA CCGTGCTGTC CAAAGGGCTG ACCTTTCGCC CGTCACTCGC

3921  CAACGCAATT AATGTGAGTT AGCTCACTCA TTAGGCACCC CAGGCTTTAC ACTTTATGCT TCCGGCTCGT
      GTTGCGTTAA TTACACTCAA TCGAGTGAGT AATCCGTGGG GTCCGAAATG TGAAATACGA AGGCCGAGCA

                                                                            BssHII
                                                                                ~~
3991  ATGTTGTGTG GAATTGTGAG CGGATAACAA TTTCACACAG GAAACAGCTA TGACCATGAT TACGCCAAGC
      TACAACACAC CTTAACACTC GCCTATTGTT AAAGTGTGTC CTTTGTCGAT ACTGGTACTA ATGCGGTTCG

      BssHII                            EcoRI
      ~~~~                                  ~~~~~~~
4061  GCGCAATTAA CCCTCACTAA AGGGAACAAA AGCTGGAATT CCACAATGAA CAATAATAAG ATTAAAATAG
      CGCGTTAATT GGGAGTGATT TCCCTTGTTT TCGACCTTAA GGTGTTACTT GTTATTATTC TAATTTTATC

4131  CTTGCCCCCG TTGCAGCGAT GGGTATTTTT TCTAGTAAAA TAAAAGATAA ACTTAGACTC AAAACATTTA
      GAACGGGGGC AACGTCGCTA CCCATAAAAA AGATCATTTT ATTTTCTATT TGAATCTGAG TTTTGTAAAT

4201  CAAAAACAAC CCCTAAAGTC CTAAAGCCCA AAGTGCTATG CACGATCCAT AGCAAGCCCA GCCCAACCCA
      GTTTTTGTTG GGGATTTCAG GATTTCGGGT TTCACGATAC GTGCTAGGTA TCGTTCGGGT CGGGTTGGGT
```

FIG._43G

```
4271  ACCCAACCCA ACCCACCCCA GTGCAGCCAA CTGGCAAATA GTCTCCACCC CCGGCACTAT CACCGTGAGT
      TGGGTTGGGT TGGGTGGGGT CACGTCGGTT GACCGTTTAT CAGAGGTGGG GGCCGTGATA GTGGCACTCA

4341  TGTCCGCACC ACCGCACGTC TCGCAGCCAA AAAAAAAAAA AGAAAGAAAA AAAAGAAAAA GAAAAACAGC
      ACAGGCGTGG TGGCGTGCAG AGCGTCGGTT TTTTTTTTTT TCTTTCTTTT TTTTCTTTTT CTTTTTGTCG

4411  AGGTGGGTCC GGGTCGTGGG GGCCGGAAAA GCGAGGAGGA TCGCGAGCAG CGACGAGGCC CGGCCCTCCC
      TCCACCCAGG CCCAGCACCC CCGGCCTTTT CGCTCCTCCT AGCGCTCGTC GCTGCTCCGG GCCGGGAGGG

4481  TCCGCTTCCA AAGAAACGCC CCCCATCGCC ACTATATACA TACCCCCCCC TCTCCTCCCA TCCCCCCAAC
      AGGCGAAGGT TTCTTTGCGG GGGGTAGCGG TGATATATGT ATGGGGGGGG AGAGGAGGGT AGGGGGGTTG

4551  CCTACCACCA CCACCACCAC CACCTCCTCC CCCCTCGCTG CCGGACGACG AGCTCCTCCC CCCTCCCCCT
      GGATGGTGGT GGTGGTGGTG GTGGAGGAGG GGGGAGCGAC GGCCTGCTGC TCGAGGAGGG GGGAGGGGGA

4621  CCGCCGCCGC CGGTAACCAC CCCGCCCCTC TCCTCTTTCT TTCTCCGTTT TTTTTTTCGT CTCGGTCTCG
      GGCGGCGGCG GCCATTGGTG GGGCGGGGAG AGGAGAAAGA AAGAGGCAAA AAAAAAAGCA GAGCCAGAGC

4691  ATCTTTGGCC TTGGTAGTTT GGGTGGGCGA GAGCGGCTTC GTCGCCCAGA TCGGTGCGCG GGAGGGGCGG
      TAGAAACCGG AACCATCAAA CCCACCCGCT CTCGCCGAAG CAGCGGGTCT AGCCACGCGC CCTCCCCGCC

                                                BamHI                       BglII
                                              ~~~~~~~~                          ~
4761  GATCTCGCGG CTGGCGTCTC CGGGCGTGAG TCGGCCCGGA TCCTCGCGGG GAATGGGGCT CTCGGATGTA
      CTAGAGCGCC GACCGCAGAG GCCCGCACTC AGCCGGGCCT AGGAGCGCCC CTTACCCCGA GAGCCTACAT

      BglII
      ~~~~~
4831  GATCTTCTTT CTTTCTTCTT TTTGTGGTAG AATTTGAATC CCTCAGCATT GTTCATCGGT AGTTTTTCTT
      CTAGAAGAAA GAAAGAAGAA AAACACCATC TTAAACTTAG GGAGTCGTAA CAAGTAGCCA TCAAAAAGAA

4901  TTCATGATTT GTGACAAATG CAGCCTCGTG CGGAGCTTTT TTGTAGGTAG
      AAGTACTAAA CACTGTTTAC GTCGGAGCAC GCCTCGAAAA AACATCCATC
```

FIG._43H

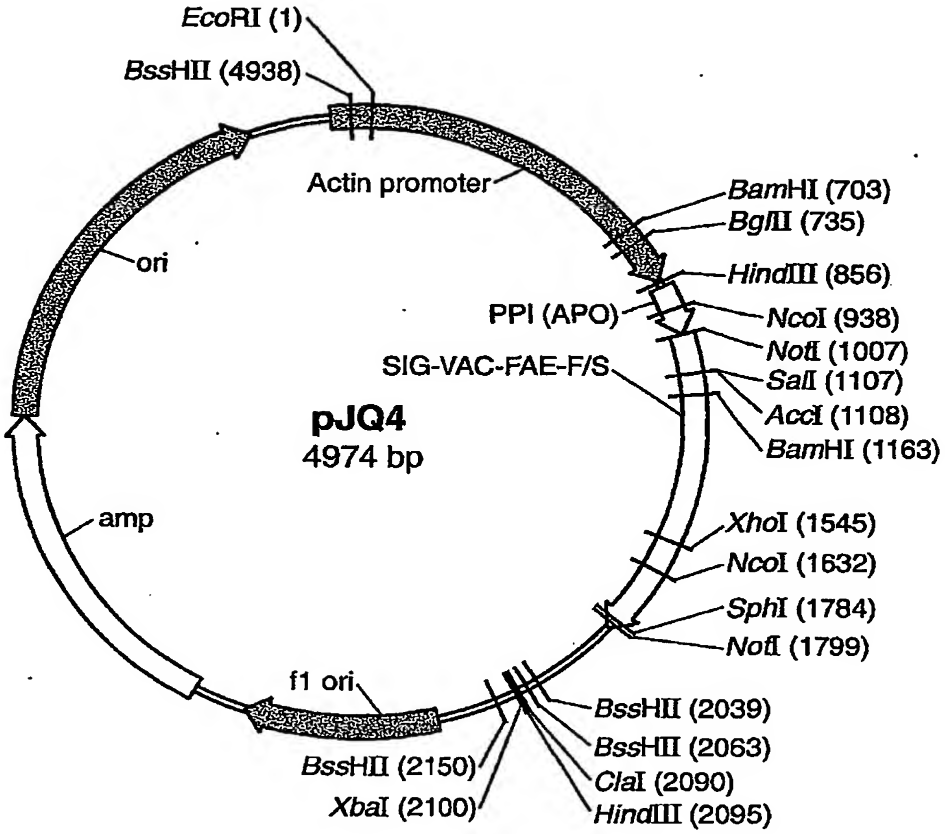

EcoRI (1)
BssHII (4938)
Actin promoter
BamHI (703)
BglII (735)
HindIII (856)
ori
PPI (APO)
NcoI (938)
NotI (1007)
SIG-VAC-FAE-F/S
SalI (1107)
AccI (1108)
BamHI (1163)
pJQ4
4974 bp
amp
XhoI (1545)
NcoI (1632)
SphI (1784)
NotI (1799)
f1 ori
BssHII (2039)
BssHII (2063)
ClaI (2090)
HindIII (2095)
BssHII (2150)
XbaI (2100)

FIG._44A

```
     EcoRI
     ~~~~~~
  1  AATTCCACAA TGAACAATAA TAAGATTAAA ATAGCTTGCC CCCGTTGCAG CGATGGGTAT TTTTTCTAGT
     TTAAGGTGTT ACTTGTTATT ATTCTAATTT TATCGAACGG GGGCAACGTC GCTACCCATA AAAAAGATCA

 71  AAAATAAAAG ATAAACTTAG ACTCAAAACA TTTACAAAAA CAACCCCTAA AGTCCTAAAG CCCAAAGTGC
     TTTTATTTTC TATTTGAATC TGAGTTTTGT AAATGTTTTT GTTGGGGATT TCAGGATTTC GGGTTTCACG

141  TATGCACGAT CCATAGCAAG CCCAGCCCAA CCCAACCCAA CCCAACCCAC CCCAGTGCAG CCAACTGGCA
     ATACGTGCTA GGTATCGTTC GGGTCGGGTT GGGTTGGGTT GGGTTGGGTG GGGTCACGTC GGTTGACCGT

211  AATAGTCTCC ACCCCCGGCA CTATCACCGT GAGTTGTCCG CACCACCGCA CGTCTCGCAG CCAAAAAAAA
     TTATCAGAGG TGGGGGCCGT GATAGTGGCA CTCAACAGGC GTGGTGGCGT GCAGAGCGTC GGTTTTTTTT

281  AAAAAGAAAG AAAAAAAAGA AAAAGAAAAA CAGCAGGTGG GTCCGGGTCG TGGGGGCCGG AAAAGCGAGG
     TTTTTCTTTC TTTTTTTTCT TTTTCTTTTT GTCGTCCACC CAGGCCCAGC ACCCCCGGCC TTTTCGCTCC

351  AGGATCGCGA GCAGCGACGA GGCCCGGCCC TCCCTCCGCT TCCAAAGAAA CGCCCCCCAT CGCCACTATA
     TCCTAGCGCT CGTCGCTGCT CCGGGCCGGG AGGGAGGCGA AGGTTTCTTT GCGGGGGGTA GCGGTGATAT

421  TACATACCCC CCCCTCTCCT CCCATCCCCC CAACCCTACC ACCACCACCA CCACCACCTC CTCCCCCCTC
     ATGTATGGGG GGGGAGAGGA GGGTAGGGGG GTTGGGATGG TGGTGGTGGT GGTGGTGGAG GAGGGGGGAG

491  GCTGCCGGAC GACGAGCTCC TCCCCCCTCC CCCTCCGCCG CCGCCGGTAA CCACCCCGCC CCTCTCCTCT
     CGACGGCCTG CTGCTCGAGG AGGGGGGAGG GGGAGGCGGC GGCGGCCATT GGTGGGGCGG GGAGAGGAGA

561  TTCTTTCTCC GTTTTTTTTT TCGTCTCGGT CTCGATCTTT GGCCTTGGTA GTTTGGGTGG GCGAGAGCGG
     AAGAAAGAGG CAAAAAAAAA AGCAGAGCCA GAGCTAGAAA CCGGAACCAT CAAACCCACC CGCTCTCGCC

631  CTTCGTCGCC CAGATCGGTG CGCGGGAGGG GCGGGATCTC GCGGCTGGCG TCTCCGGGCG TGAGTCGGCC
     GAAGCAGCGG GTCTAGCCAC GCGCCCTCCC CGCCCTAGAG CGCCGACCGC AGAGGCCCGC ACTCAGCCGG

       BamHI                             BglII
      ~~~~~~~                           ~~~~~~~
701  CGGATCCTCG CGGGGAATGG GGCTCTCGGA TGTAGATCTT CTTTCTTTCT TCTTTTTGTG GTAGAATTTG
     GCCTAGGAGC GCCCCTTACC CCGAGAGCCT ACATCTAGAA GAAAGAAAGA AGAAAAACAC CATCTTAAAC
```

FIG._44B

```
 771 AATCCCTCAG CATTGTTCAT CGGTAGTTTT TCTTTTCATG ATTTGTGACA AATGCAGCCT CGTGCGGAGC
     TTAGGGAGTC GTAACAAGTA GCCATCAAAA AGAAAAGTAC TAAACACTGT TTACGTCGGA GCACGCCTCG

                   HindIII
                    ~~~~~~
 841 TTTTTTGTAG GTAGAAGCTT ACMATGGMCG TGCACAAGGA GGTSAACTTC GTSGCCTACC TCCTGATCGT
     AAAAAACATC CATCTTCGAA TGKTACCKGC ACGTGTTCCT CCASTTGAAG CASCGGATGG AGGACTAGCA

                                  NcoI
                                 ~~~~~~~
 911 SCTCGGCCTC CTCTTGCTCG TSTCCGCCAT GGAGCACGTG GACGCCAAGG CCTGCACCCK CGAGTGCGGC
     SGAGCCGGAG GAGAACGAGC ASAGGCGGTA CCTCGTGCAC CTGCGGTTCC GGACGTGGGM GCTCACGCCG

                                 NotI
                               ~~~~~~~~~
 981 AACCTCGGCT TCGGCATCTG CCCGGCGGCC GCCTCCACGC AGGGCATCTC CGAAGACCTC TACAGCCGTT
     TTGGAGCCGA AGCCGTAGAC GGGCCGCCGG CGGAGGTGCG TCCCGTAGAG GCTTCTGGAG ATGTCGGCAA

                                                                  SalI
                                                                 ~~~~~~~
                                                                  AccI
                                                                 ~~~~~~~
1051 TAGTCGAAAT GGCCACTATC TCCCAAGCTG CCTACGCCGA CCTGTGCAAC ATTCCGTCGA CTATTATCAA
     ATCAGCTTTA CCGGTGATAG AGGGTTCGAC GGATGCGGCT GGACACGTTG TAAGGCAGCT GATAATAGTT

                                                  BamHI
                                                  ~~~~~~
1121 GGGAGAGAAA ATTTACAATT CTCAAACTGA CATTAACGGA TGGATCCTCC GCGACGACAG CAGCAAAGAA
     CCCTCTCTTT TAAATGTTAA GAGTTTGACT GTAATTGCCT ACCTAGGAGG CGCTGCTGTC GTCGTTTCTT

1191 ATAATCACCG TCTTCCGTGG CACTGGTAGT GATACGAATC TACAACTCGA TACTAACTAC ACCCTCACGC
     TATTAGTGGC AGAAGGCACC GTGACCATCA CTATGCTTAG ATGTTGAGCT ATGATTGATG TGGGAGTGCG

1261 CTTTCGACAC CCTACCACAA TGCAACGGTT GTGAAGTACA CGGTGGATAT TATATTGGAT GGGTCTCCGT
     GAAAGCTGTG GGATGGTGTT ACGTTGCCAA CACTTCATGT GCCACCTATA ATATAACCTA CCCAGAGGCA
```

FIG._44C

```
1331   CCAGGACCAA GTCGAGTCGC TTGTCAAACA GCAGGTTAGC CAGTATCCGG ACTACGCGCT GACCGTGACC
       GGTCCTGGTT CAGCTCAGCG AACAGTTTGT CGTCCAATCG GTCATAGGCC TGATGCGCGA CTGGCACTGG

1401   GGCCACKCCC TCGGCGCCTC CCTGGCGGCA CTCACTGCCG CCCAGCTGTC TGCGACATAC GACAACATCC
       CCGGTGMGGG AGCCGCGGAG GGACCGCCGT GAGTGACGGC GGGTCGACAG ACGCTGTATG CTGTTGTAGG

1471   GCCTGTACAC CTTCGGCGAA CCGCGCAGCG GCAATCAGGC CTTCGCGTCG TACATGAACG ATGCCTTCCA
       CGGACATGTG GAAGCCGCTT GGCGCGTCGC CGTTAGTCCG GAAGCGCAGC ATGTACTTGC TACGGAAGGT

           XhoI
          ~~~~~~
1541   AGCCTCGAGC CCAGATACGA CGCAGTATTT CCGGGTCACT CATGCCAACG ACGGCATCCC AAACCTGCCC
       TCGGAGCTCG GGTCTATGCT GCGTCATAAA GGCCCAGTGA GTACGGTTGC TGCCGTAGGG TTTGGACGGG

                              NcoI
                             ~~~~~~
1611   CCGGTGGAGC AGGGGTACGC CCATGGCGGT GTAGAGTACT GGAGCGTTGA TCCTTACAGC GCCCAGAACA
       GGCCACCTCG TCCCCATGCG GGTACCGCCA CATCTCATGA CCTCGCAACT AGGAATGTCG CGGGTCTTGT

1681   CATTTGTCTG CACTGGGGAT GAAGTGCAGT GCTGTGAGGC CCAGGGCGGA CAGGGTGTGA ATAATGCGCA
       GTAAACAGAC GTGACCCCTA CTTCACGTCA CGACACTCCG GGTCCCGCCT GTCCCACACT TATTACGCGT

                                      SphI                NotI
                                     ~~~~~~~~           ~~~~~~~~~~
1751   CACGACTTAT TTTGGGATGA CGAGCGGCGC ATGCACCTGG CCGGTCGCGG CCGCGGAAAC CACTGAAGGA
       GTGCTGAATA AAACCCTACT GCTCGCCGCG TACGTGGACC GGCCAGCGCC GGCGCCTTTG GTGACTTCCT

1821   TGAGCTGTAA AGAAGCAGAT CGTTCAAACA TTTGGCAATA AAGTTTCTTA AGATTGAATC CTGTTGCCGG
       ACTCGACATT TCTTCGTCTA GCAAGTTTGT AAACCGTTAT TTCAAAGAAT TCTAACTTAG GACAACGGCC

1891   TCTTGCGATG ATTATCATAT AATTTCTGTT GAATTACGTT AAGCATGTAA TAATTAACAT GTAATGCATG
       AGAACGCTAC TAATAGTATA TTAAAGACAA CTTAATGCAA TTCGTACATT ATTAATTGTA CATTACGTAC

1961   ACGTTATTTA TGAGATGGGT TTTTATGATT AGAGTCCCGC AATTATACAT TTAATACGCG ATAGAAAACA
       TGCAATAAAT ACTCTACCCA AAAATACTAA TCTCAGGGCG TTAATATGTA AATTATGCGC TATCTTTTGT
```

FIG._44D

```
                                                                             XbaI
                                                                              ~~
             BssHII                     BssHII                      ClaI  HindIII
             ~~~~~~~~                   ~~~~~~~                    ~~~~~~~~~~~~~
2031  AAATATAGCG CGCAAACTAG GATAAATTAT CGCGCGCGGT GTCATCTATG TTACTAGATC GATAAGCTTC
      TTTATATCGC GCGTTTGATC CTATTTAATA GCGCGCGCCA CAGTAGATAC AATGATCTAG CTATTCGAAG

      XbaI                                               BssHII
      ~~~~                                                ~~~~~~~
2101  TAGAGCGGCC GGTGGAGCTC CAATTCGCCC TATAGTGAGT CGTATTACGC GCGCTCACTG GCCGTCGTTT
      ATCTCGCCGG CCACCTCGAG GTTAAGCGGG ATATCACTCA GCATAATGCG CGCGAGTGAC CGGCAGCAAA

2171  TACAACGTCG TGACTGGGAA AACCCTGGCG TTACCCAACT TAATCGCCTT GCAGCACATC CCCCTTTCGC
      ATGTTGCAGC ACTGACCCTT TTGGGACCGC AATGGGTTGA ATTAGCGGAA CGTCGTGTAG GGGGAAAGCG

2241  CAGCTGGCGT AATAGCGAAG AGGCCCGCAC CGATCGCCCT TCCCAACAGT TGCGCAGCCT GAATGGCGAA
      GTCGACCGCA TTATCGCTTC TCCGGGCGTG GCTAGCGGGA AGGGTTGTCA ACGCGTCGGA CTTACCGCTT

2311  TGGGACGCGC CCTGTAGCGG CGCATTAAGC GCGGCGGGTG TGGTGGTTAC GCGCAGCGTG ACCGCTACAC
      ACCCTGCGCG GGACATCGCC GCGTAATTCG CGCCGCCCAC ACCACCAATG CGCGTCGCAC TGGCGATGTG

2381  TTGCCAGCGC CCTAGCGCCC GCTCCTTTCG CTTTCTTCCC TTCCTTTCTC GCCACGTTCG CCGGCTTTCC
      AACGGTCGCG GGATCGCGGG CGAGGAAAGC GAAAGAAGGG AAGGAAAGAG CGGTGCAAGC GGCCGAAAGG

2451  CCGTCAAGCT CTAAATCGGG GGCTCCCTTT AGGGTTCCGA TTTAGTGCTT TACGGCACCT CGACCCCAAA
      GGCAGTTCGA GATTTAGCCC CCGAGGGAAA TCCCAAGGCT AAATCACGAA ATGCCGTGGA GCTGGGGTTT

2521  AAACTTGATT AGGGTGATGG TTCACGTAGT GGGCCATCGC CCTGATAGAC GGTTTTTCGC CCTTTGACGT
      TTTGAACTAA TCCCACTACC AAGTGCATCA CCCGGTAGCG GGACTATCTG CCAAAAAGCG GGAAACTGCA

2591  TGGAGTCCAC GTTCTTTAAT AGTGGACTCT TGTTCCAAAC TGGAACAACA CTCAACCCTA TCTCGGTCTA
      ACCTCAGGTG CAAGAAATTA TCACCTGAGA ACAAGGTTTG ACCTTGTTGT GAGTTGGGAT AGAGCCAGAT

2661  TTCTTTTGAT TTATAAGGGA TTTTGCCGAT TTCGGCCTAT TGGTTAAAAA ATGAGCTGAT TTAACAAAAA
      AAGAAAACTA AATATTCCCT AAAACGGCTA AAGCCGGATA ACCAATTTTT TACTCGACTA AATTGTTTTT
```

FIG._44E

```
2731  TTTAACGCGA ATTTTAACAA AATATTAACG CTTACAATTT AGGTGGCACT TTTCGGGGAA ATGTGCGCGG
      AAATTGCGCT TAAAATTGTT TTATAATTGC GAATGTTAAA TCCACCGTGA AAAGCCCCTT TACACGCGCC

2801  AACCCCTATT TGTTTATTTT TCTAAATACA TTCAAATATG TATCCGCTCA TGAGACAATA ACCCTGATAA
      TTGGGGATAA ACAAATAAAA AGATTTATGT AAGTTTATAC ATAGGCGAGT ACTCTGTTAT TGGGACTATT

2871  ATGCTTCAAT AATATTGAAA AAGGAAGAGT ATGAGTATTC AACATTTCCG TGTCGCCCTT ATTCCCTTTT
      TACGAAGTTA TTATAACTTT TTCCTTCTCA TACTCATAAG TTGTAAAGGC ACAGCGGGAA TAAGGGAAAA

2941  TTGCGGCATT TTGCCTTCCT GTTTTTGCTC ACCCAGAAAC GCTGGTGAAA GTAAAAGATG CTGAAGATCA
      AACGCCGTAA AACGGAAGGA CAAAAACGAG TGGGTCTTTG CGACCACTTT CATTTTCTAC GACTTCTAGT

3011  GTTGGGTGCA CGAGTGGGTT ACATCGAACT GGATCTCAAC AGCGGTAAGA TCCTTGAGAG TTTTCGCCCC
      CAACCCACGT GCTCACCCAA TGTAGCTTGA CCTAGAGTTG TCGCCATTCT AGGAACTCTC AAAAGCGGGG

3081  GAAGAACGTT TTCCAATGAT GAGCACTTTT AAAGTTCTGC TATGTGGCGC GGTATTATCC CGTATTGACG
      CTTCTTGCAA AAGGTTACTA CTCGTGAAAA TTTCAAGACG ATACACCGCG CCATAATAGG GCATAACTGC

3151  CCGGGCAAGA GCAACTCGGT CGCCGCATAC ACTATTCTCA GAATGACTTG GTTGAGTACT CACCAGTCAC
      GGCCCGTTCT CGTTGAGCCA GCGGCGTATG TGATAAGAGT CTTACTGAAC CAACTCATGA GTGGTCAGTG

3221  AGAAAAGCAT CTTACGGATG GCATGACAGT AAGAGAATTA TGCAGTGCTG CCATAACCAT GAGTGATAAC
      TCTTTTCGTA GAATGCCTAC CGTACTGTCA TTCTCTTAAT ACGTCACGAC GGTATTGGTA CTCACTATTG

3291  ACTGCGGCCA ACTTACTTCT GACAACGATC GGAGGACCGA AGGAGCTAAC CGCTTTTTTG CACAACATGG
      TGACGCCGGT TGAATGAAGA CTGTTGCTAG CCTCCTGGCT TCCTCGATTG GCGAAAAAAC GTGTTGTACC

3361  GGGATCATGT AACTCGCCTT GATCGTTGGG AACCGGAGCT GAATGAAGCC ATACCAAACG ACGAGCGTGA
      CCCTAGTACA TTGAGCGGAA CTAGCAACCC TTGGCCTCGA CTTACTTCGG TATGGTTTGC TGCTCGCACT

3431  CACCACGATG CCTGTAGCAA TGGCAACAAC GTTGCGCAAA CTATTAACTG GCGAACTACT TACTCTAGCT
      GTGGTGCTAC GGACATCGTT ACCGTTGTTG CAACGCGTTT GATAATTGAC CGCTTGATGA ATGAGATCGA

3501  TCCCGGCAAC AATTAATAGA CTGGATGGAG GCGGATAAAG TTGCAGGACC ACTTCTGCGC TCGGCCCTTC
      AGGGCCGTTG TTAATTATCT GACCTACCTC CGCCTATTTC AACGTCCTGG TGAAGACGCG AGCCGGGAAG
```

FIG._44F

```
3571  CGGCTGGCTG GTTTATTGCT GATAAATCTG GAGCCGGTGA GCGTGGGTCT CGCGGTATCA TTGCAGCACT
      GCCGACCGAC CAAATAACGA CTATTTAGAC CTCGGCCACT CGCACCCAGA GCGCCATAGT AACGTCGTGA

3641  GGGGCCAGAT GGTAAGCCCT CCCGTATCGT AGTTATCTAC ACGACGGGGA GTCAGGCAAC TATGGATGAA
      CCCCGGTCTA CCATTCGGGA GGGCATAGCA TCAATAGATG TGCTGCCCCT CAGTCCGTTG ATACCTACTT

3711  CGAAATAGAC AGATCGCTGA GATAGGTGCC TCACTGATTA AGCATTGGTA ACTGTCAGAC CAAGTTTACT
      GCTTTATCTG TCTAGCGACT CTATCCACGG AGTGACTAAT TCGTAACCAT TGACAGTCTG GTTCAAATGA

3781  CATATATACT TTAGATTGAT TTAAAACTTC ATTTTTAATT TAAAAGGATC TAGGTGAAGA TCCTTTTTGA
      GTATATATGA AATCTAACTA AATTTTGAAG TAAAAATTAA ATTTTCCTAG ATCCACTTCT AGGAAAAACT

3851  TAATCTCATG ACCAAAATCC CTTAACGTGA GTTTTCGTTC CACTGAGCGT CAGACCCCGT AGAAAAGATC
      ATTAGAGTAC TGGTTTTAGG GAATTGCACT CAAAAGCAAG GTGACTCGCA GTCTGGGGCA TCTTTTCTAG

3921  AAAGGATCTT CTTGAGATCC TTTTTTTCTG CGCGTAATCT GCTGCTTGCA AACAAAAAAA CCACCGCTAC
      TTTCCTAGAA GAACTCTAGG AAAAAAAGAC GCGCATTAGA CGACGAACGT TTGTTTTTTT GGTGGCGATG

3991  CAGCGGTGGT TTGTTTGCCG GATCAAGAGC TACCAACTCT TTTTCCGAAG GTAACTGGCT TCAGCAGAGC
      GTCGCCACCA AACAAACGGC CTAGTTCTCG ATGGTTGAGA AAAAGGCTTC CATTGACCGA AGTCGTCTCG

4061  GCAGATACCA AATACTGTCC TTCTAGTGTA GCCGTAGTTA GGCCACCACT TCAAGAACTC TGTAGCACCG
      CGTCTATGGT TTATGACAGG AAGATCACAT CGGCATCAAT CCGGTGGTGA AGTTCTTGAG ACATCGTGGC

4131  CCTACATACC TCGCTCTGCT AATCCTGTTA CCAGTGGCTG CTGCCAGTGG CGATAAGTCG TGTCTTACCG
      GGATGTATGG AGCGAGACGA TTAGGACAAT GGTCACCGAC GACGGTCACC GCTATTCAGC ACAGAATGGC

4201  GGTTGGACTC AAGACGATAG TTACCGGATA AGGCGCAGCG GTCGGGCTGA ACGGGGGGTT CGTGCACACA
      CCAACCTGAG TTCTGCTATC AATGGCCTAT TCCGCGTCGC CAGCCCGACT TGCCCCCCAA GCACGTGTGT

4271  GCCCAGCTTG GAGCGAACGA CCTACACCGA ACTGAGATAC CTACAGCGTG AGCTATGAGA AAGCGCCACG
      CGGGTCGAAC CTCGCTTGCT GGATGTGGCT TGACTCTATG GATGTCGCAC TCGATACTCT TTCGCGGTGC

4341  CTTCCCGAAG GGAGAAAGGC GGACAGGTAT CCGGTAAGCG GCAGGGTCGG AACAGGAGAG CGCACGAGGG
      GAAGGGCTTC CCTCTTTCCG CCTGTCCATA GGCCATTCGC CGTCCCAGCC TTGTCCTCTC GCGTGCTCCC
```

FIG._44G

```
4411  AGCTTCCAGG GGGAAACGCC TGGTATCTTT ATAGTCCTGT CGGGTTTCGC CACCTCTGAC TTGAGCGTCG
      TCGAAGGTCC CCCTTTGCGG ACCATAGAAA TATCAGGACA GCCCAAAGCG GTGGAGACTG AACTCGCAGC

4481  ATTTTTGTGA TGCTCGTCAG GGGGGCGGAG CCTATGGAAA AACGCCAGCA ACGCGGCCTT TTTACGGTTC
      TAAAAACACT ACGAGCAGTC CCCCCGCCTC GGATACCTTT TTGCGGTCGT TGCGCCGGAA AAATGCCAAG

4551  CTGGCCTTTT GCTGGCCTTT TGCTCACATG TTCTTTCCTG CGTTATCCCC TGATTCTGTG GATAACCGTA
      GACCGGAAAA CGACCGGAAA ACGAGTGTAC AAGAAAGGAC GCAATAGGGG ACTAAGACAC CTATTGGCAT

4621  TTACCGCCTT TGAGTGAGCT GATACCGCTC GCCGCAGCCG AACGACCGAG CGCAGCGAGT CAGTGAGCGA
      AATGGCGGAA ACTCACTCGA CTATGGCGAG CGGCGTCGGC TTGCTGGCTC GCGTCGCTCA GTCACTCGCT

4691  GGAAGCGGAA GAGCGCCCAA TACGCAAACC GCCTCTCCCC GCGCGTTGGC CGATTCATTA ATGCAGCTGG
      CCTTCGCCTT CTCGCGGGTT ATGCGTTTGG CGGAGAGGGG CGCGCAACCG GCTAAGTAAT TACGTCGACC

4761  CACGACAGGT TTCCCGACTG GAAAGCGGGC AGTGAGCGCA ACGCAATTAA TGTGAGTTAG CTCACTCATT
      GTGCTGTCCA AAGGGCTGAC CTTTCGCCCG TCACTCGCGT TGCGTTAATT ACACTCAATC GAGTGAGTAA

4831  AGGCACCCCA GGCTTTACAC TTTATGCTTC CGGCTCGTAT GTTGTGTGGA ATTGTGAGCG GATAACAATT
      TCCGTGGGGT CCGAAATGTG AAATACGAAG GCCGAGCATA CAACACACCT TAACACTCGC CTATTGTTAA

                                           BssHII
                                           ~~~~~~~
4901  TCACACAGGA AACAGCTATG ACCATGATTA CGCCAAGCGC GCAATTAACC CTCACTAAAG GGAACAAAAG
      AGTGTGTCCT TTGTCGATAC TGGTACTAAT GCGGTTCGCG CGTTAATTGG GAGTGATTTC CCTTGTTTTC

      EcoR

4971  CTGG
      GACC
```

FIG._44H

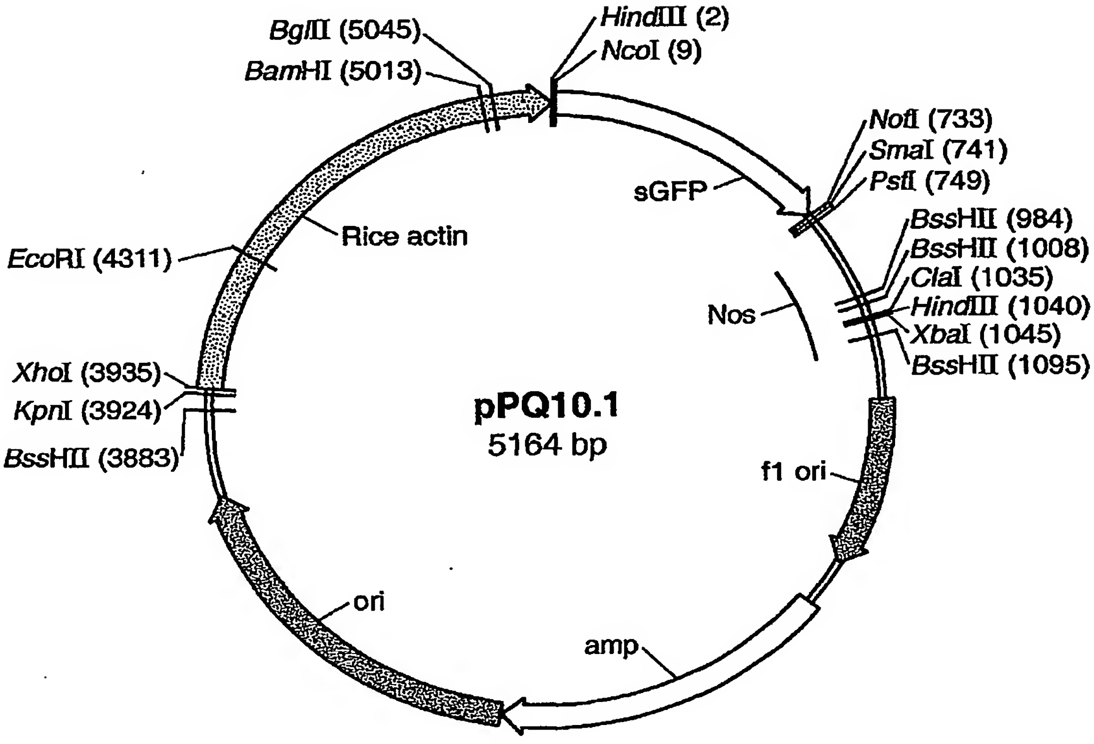

BglII (5045)
BamHI (5013)
HindIII (2)
NcoI (9)
NotI (733)
SmaI (741)
PstI (749)
sGFP
BssHII (984)
BssHII (1008)
ClaI (1035)
HindIII (1040)
XbaI (1045)
BssHII (1095)
Nos
Rice actin
EcoRI (4311)
XhoI (3935)
KpnI (3924)
BssHII (3883)
pPQ10.1
5164 bp
f1 ori
ori
amp

FIG._45A

Sequence for pPQ10.1

```
          HindIII NcoI
          ~~~~~~~ ~~~~~~~~
       1  AAGCTTACCA TGGTGAGCAA GGGCGAGGAG CTGTTCACCG GGGTGGTGCC
CATCCTGGTC GAGCTGGACG
          TTCGAATGGT ACCACTCGTT CCCGCTCCTC GACAAGTGGC CCCACCACGG
GTAGGACCAG CTCGACCTGC

      71  GCGACGTGAA CGGCCACAAG TTCAGCGTGT CCGGCGAGGG CGAGGGCGAT
GCCACCTACG GCAAGCTGAC
          CGCTGCACTT GCCGGTGTTC AAGTCGCACA GGCCGCTCCC GCTCCCGCTA
CGGTGGATGC CGTTCGACTG

     141  CCTGAAGTTC ATCTGCACCA CCGGCAAGCT GCCCGTGCCC TGGCCCACCC
TCGTGACCAC CTTCACCTAC
          GGACTTCAAG TAGACGTGGT GGCCGTTCGA CGGGCACGGG ACCGGGTGGG
AGCACTGGTG GAAGTGGATG

     211  GGCGTGCAGT GCTTCAGCCG CTACCCCGAC CACATGAAGC AGCACGACTT
CTTCAAGTCC GCCATGCCCG
          CCGCACGTCA CGAAGTCGGC GATGGGGCTG GTGTACTTCG TCGTGCTGAA
GAAGTTCAGG CGGTACGGGC

     281  AAGGCTACGT CCAGGAGCGC ACCATCTTCT TCAAGGACGA CGGCAACTAC
AAGACCCGCG CCGAGGTGAA
          TTCCGATGCA GGTCCTCGCG TGGTAGAAGA AGTTCCTGCT GCCGTTGATG
TTCTGGGCGC GGCTCCACTT

     351  GTTCGAGGGC GACACCCTGG TGAACCGCAT CGAGCTGAAG GGCATCGACT
TCAAGGAGGA CGGCAACATC
          CAAGCTCCCG CTGTGGGACC ACTTGGCGTA GCTCGACTTC CCGTAGCTGA
AGTTCCTCCT GCCGTTGTAG

     421  CTGGGGCACA AGCTGGAGTA CAACTACAAC AGCCACAACG TCTATATCAT
GGCCGACAAG CAGAAGAACG
          GACCCCGTGT TCGACCTCAT GTTGATGTTG TCGGTGTTGC AGATATAGTA
CCGGCTGTTC GTCTTCTTGC

     491  GCATCAAGGT GAACTTCAAG ATCCGCCACA ACATCGAGGA CGGCAGCGTG
CAGCTCGCCG ACCACTACCA
          CGTAGTTCCA CTTGAAGTTC TAGGCGGTGT TGTAGCTCCT GCCGTCGCAC
GTCGAGCGGC TGGTGATGGT

     561  GCAGAACACC CCCATCGGCG ACGGCCCCGT GCTGCTGCCC GACAACCACT
ACCTGAGCAC CCAGTCCGCC
          CGTCTTGTGG GGGTAGCCGC TGCCGGGGCA CGACGACGGG CTGTTGGTGA
TGGACTCGTG GGTCAGGCGG

     631  CTGAGCAAAG ACCCCAACGA GAAGCGCGAT CACATGGTCC TGCTGGAGTT
CGTGACCGCC GCCGGGATCA
```

Fig. 45B

```
            CCGCATTATC GCTTCTCCGG GCGTGGCTAG CGGGAAGGGT TGTCAACGCG
TCGGACTTAC CGCTTACCCT

     1261   CGCGCCCTGT AGCGGCGCAT TAAGCGCGGC GGGTGTGGTG GTTACGCGCA
GCGTGACCGC TACACTTGCC
            GCGCGGGACA TCGCCGCGTA ATTCGCGCCG CCCACACCAC CAATGCGCGT
CGCACTGGCG ATGTGAACGG

     1331   AGCGCCCTAG CGCCCGCTCC TTTCGCTTTC TTCCCTTCCT TTCTCGCCAC
GTTCGCCGGC TTTCCCCGTC
            TCGCGGGATC GCGGGCGAGG AAAGCGAAAG AAGGGAAGGA AAGAGCGGTG
CAAGCGGCCG AAAGGGGCAG

     1401   AAGCTCTAAA TCGGGGGCTC CCTTTAGGGT TCCGATTTAG TGCTTTACGG
CACCTCGACC CCAAAAAACT
            TTCGAGATTT AGCCCCCGAG GGAAATCCCA AGGCTAAATC ACGAAATGCC
GTGGAGCTGG GGTTTTTTGA

     1471   TGATTAGGGT GATGGTTCAC GTAGTGGGCC ATCGCCCTGA TAGACGGTTT
TTCGCCCTTT GACGTTGGAG
            ACTAATCCCA CTACCAAGTG CATCACCCGG TAGCGGGACT ATCTGCCAAA
AAGCGGGAAA CTGCAACCTC

     1541   TCCACGTTCT TTAATAGTGG ACTCTTGTTC CAAACTGGAA CAACACTCAA
CCCTATCTCG GTCTATTCTT
            AGGTGCAAGA AATTATCACC TGAGAACAAG GTTTGACCTT GTTGTGAGTT
GGGATAGAGC CAGATAAGAA

     1611   TTGATTTATA AGGGATTTTG CCGATTTCGG CCTATTGGTT AAAAAATGAG
CTGATTTAAC AAAAATTTAA
            AACTAAATAT TCCCTAAAAC GGCTAAAGCC GGATAACCAA TTTTTTACTC
GACTAAATTG TTTTTAAATT

     1681   CGCGAATTTT AACAAAATAT TAACGCTTAC AATTTAGGTG GCACTTTTCG
GGGAAATGTG CGCGGAACCC
            GCGCTTAAAA TTGTTTTATA ATTGCGAATG TTAAATCCAC CGTGAAAAGC
CCCTTTACAC GCGCCTTGGG

     1751   CTATTTGTTT ATTTTTCTAA ATACATTCAA ATATGTATCC GCTCATGAGA
CAATAACCCT GATAAATGCT
            GATAAACAAA TAAAAAGATT TATGTAAGTT TATACATAGG CGAGTACTCT
GTTATTGGGA CTATTTACGA

     1821   TCAATAATAT TGAAAAAGGA AGAGTATGAG TATTCAACAT TTCCGTGTCG
CCCTTATTCC CTTTTTTGCG
            AGTTATTATA ACTTTTTCCT TCTCATACTC ATAAGTTGTA AAGGCACAGC
GGGAATAAGG GAAAAAACGC

     1891   GCATTTTGCC TTCCTGTTTT TGCTCACCCA GAAACGCTGG TGAAAGTAAA
AGATGCTGAA GATCAGTTGG
            CGTAAAACGG AAGGACAAAA ACGAGTGGGT CTTTGCGACC ACTTTCATTT
TCTACGACTT CTAGTCAACC
```

Fig. 45C

1961 GTGCACGAGT GGGTTACATC GAACTGGATC TCAACAGCGG TAAGATCCTT
GAGAGTTTTC GCCCCGAAGA
CACGTGCTCA CCCAATGTAG CTTGACCTAG AGTTGTCGCC ATTCTAGGAA
CTCTCAAAAG CGGGGCTTCT

2031 ACGTTTTCCA ATGATGAGCA CTTTTAAAGT TCTGCTATGT GGCGCGGTAT
TATCCCGTAT TGACGCCGGG
TGCAAAAGGT TACTACTCGT GAAAATTTCA AGACGATACA CCGCGCCATA
ATAGGGCATA ACTGCGGCCC

2101 CAAGAGCAAC TCGGTCGCCG CATACACTAT TCTCAGAATG ACTTGGTTGA
GTACTCACCA GTCACAGAAA
GTTCTCGTTG AGCCAGCGGC GTATGTGATA AGAGTCTTAC TGAACCAACT
CATGAGTGGT CAGTGTCTTT

2171 AGCATCTTAC GGATGGCATG ACAGTAAGAG AATTATGCAG TGCTGCCATA
ACCATGAGTG ATAACACTGC
TCGTAGAATG CCTACCGTAC TGTCATTCTC TTAATACGTC ACGACGGTAT
TGGTACTCAC TATTGTGACG

2241 GGCCAACTTA CTTCTGACAA CGATCGGAGG ACCGAAGGAG CTAACCGCTT
TTTTGCACAA CATGGGGGAT
CCGGTTGAAT GAAGACTGTT GCTAGCCTCC TGGCTTCCTC GATTGGCGAA
AAAACGTGTT GTACCCCCTA

2311 CATGTAACTC GCCTTGATCG TTGGGAACCG GAGCTGAATG AAGCCATACC
AAACGACGAG CGTGACACCA
GTACATTGAG CGGAACTAGC AACCCTTGGC CTCGACTTAC TTCGGTATGG
TTTGCTGCTC GCACTGTGGT

2381 CGATGCCTGT AGCAATGGCA ACAACGTTGC GCAAACTATT AACTGGCGAA
CTACTTACTC TAGCTTCCCG
GCTACGGACA TCGTTACCGT TGTTGCAACG CGTTTGATAA TTGACCGCTT
GATGAATGAG ATCGAAGGGC

2451 GCAACAATTA ATAGACTGGA TGGAGGCGGA TAAAGTTGCA GGACCACTTC
TGCGCTCGGC CCTTCCGGCT
CGTTGTTAAT TATCTGACCT ACCTCCGCCT ATTTCAACGT CCTGGTGAAG
ACGCGAGCCG GGAAGGCCGA

2521 GGCTGGTTTA TTGCTGATAA ATCTGGAGCC GGTGAGCGTG GGTCTCGCGG
TATCATTGCA GCACTGGGGC
CCGACCAAAT AACGACTATT TAGACCTCGG CCACTCGCAC CCAGAGCGCC
ATAGTAACGT CGTGACCCCG

2591 CAGATGGTAA GCCCTCCCGT ATCGTAGTTA TCTACACGAC GGGGAGTCAG
GCAACTATGG ATGAACGAAA
GTCTACCATT CGGGAGGGCA TAGCATCAAT AGATGTGCTG CCCCTCAGTC
CGTTGATACC TACTTGCTTT

2661 TAGACAGATC GCTGAGATAG GTGCCTCACT GATTAAGCAT TGGTAACTGT
CAGACCAAGT TTACTCATAT
ATCTGTCTAG CGACTCTATC CACGGAGTGA CTAATTCGTA ACCATTGACA
GTCTGGTTCA AATGAGTATA

Fig. 45D

```
2731  ATACTTTAGA TTGATTTAAA ACTTCATTTT TAATTTAAAA GGATCTAGGT GAAGATCCTT TTTGATAATC
      TATGAAATCT AACTAAATTT TGAAGTAAAA ATTAAATTTT CCTAGATCCA CTTCTAGGAA AAACTATTAG

2801  TCATGACCAA AATCCCTTAA CGTGAGTTTT CGTTCCACTG AGCGTCAGAC CCCGTAGAAA AGATCAAAGG
      AGTACTGGTT TTAGGGAATT GCACTCAAAA GCAAGGTGAC TCGCAGTCTG GGGCATCTTT TCTAGTTTCC

2871  ATCTTCTTGA GATCCTTTTT TTCTGCGCGT AATCTGCTGC TTGCAAACAA AAAAACCACC GCTACCAGCG
      TAGAAGAACT CTAGGAAAAA AAGACGCGCA TTAGACGACG AACGTTTGTT TTTTTGGTGG CGATGGTCGC

2941  GTGGTTTGTT TGCCGGATCA AGAGCTACCA ACTCTTTTTC CGAAGGTAAC TGGCTTCAGC AGAGCGCAGA
      CACCAAACAA ACGGCCTAGT TCTCGATGGT TGAGAAAAAG GCTTCCATTG ACCGAAGTCG TCTCGCGTCT

3011  TACCAAATAC TGTCCTTCTA GTGTAGCCGT AGTTAGGCCA CCACTTCAAG AACTCTGTAG CACCGCCTAC
      ATGGTTTATG ACAGGAAGAT CACATCGGCA TCAATCCGGT GGTGAAGTTC TTGAGACATC GTGGCGGATG

3081  ATACCTCGCT CTGCTAATCC TGTTACCAGT GGCTGCTGCC AGTGGCGATA AGTCGTGTCT TACCGGGTTG
      TATGGAGCGA GACGATTAGG ACAATGGTCA CCGACGACGG TCACCGCTAT TCAGCACAGA ATGGCCCAAC

3151  GACTCAAGAC GATAGTTACC GGATAAGGCG CAGCGGTCGG GCTGAACGGG GGGTTCGTGC ACACAGCCCA
      CTGAGTTCTG CTATCAATGG CCTATTCCGC GTCGCCAGCC CGACTTGCCC CCCAAGCACG TGTGTCGGGT

3221  GCTTGGAGCG AACGACCTAC ACCGAACTGA GATACCTACA GCGTGAGCTA TGAGAAAGCG CCACGCTTCC
      CGAACCTCGC TTGCTGGATG TGGCTTGACT CTATGGATGT CGCACTCGAT ACTCTTTCGC GGTGCGAAGG

3291  CGAAGGGAGA AAGGCGGACA GGTATCCGGT AAGCGGCAGG GTCGGAACAG GAGAGCGCAC GAGGGAGCTT
      GCTTCCCTCT TTCCGCCTGT CCATAGGCCA TTCGCCGTCC CAGCCTTGTC CTCTCGCGTG CTCCCTCGAA

3361  CCAGGGGGAA ACGCCTGGTA TCTTTATAGT CCTGTCGGGT TTCGCCACCT CTGACTTGAG CGTCGATTTT
      GGTCCCCCTT TGCGGACCAT AGAAATATCA GGACAGCCCA AAGCGGTGGA GACTGAACTC GCAGCTAAAA

3431  TGTGATGCTC GTCAGGGGGG CGGAGCCTAT GGAAAAACGC CAGCAACGCG GCCTTTTTAC GGTTCCTGGC
      ACACTACGAG CAGTCCCCCC GCCTCGGATA CCTTTTTGCG GTCGTTGCGC CGGAAAAATG CCAAGGACCG
```

FIG._45E

```
3501  CTTTTGCTGG CCTTTTGCTC ACATGTTCTT TCCTGCGTTA TCCCCTGATT CTGTGGATAA CCGTATTACC
      GAAAACGACC GGAAAACGAG TGTACAAGAA AGGACGCAAT AGGGGACTAA GACACCTATT GGCATAATGG

3571  GCCTTTGAGT GAGCTGATAC CGCTCGCCGC AGCCGAACGA CCGAGCGCAG CGAGTCAGTG AGCGAGGAAG
      CGGAAACTCA CTCGACTATG GCGAGCGGCG TCGGCTTGCT GGCTCGCGTC GCTCAGTCAC TCGCTCCTTC

3641  CGGAAGAGCG CCCAATACGC AAACCGCCTC TCCCCGCGCG TTGGCCGATT CATTAATGCA GCTGGCACGA
      GCCTTCTCGC GGGTTATGCG TTTGGCGGAG AGGGGCGCGC AACCGGCTAA GTAATTACGT CGACCGTGCT

3711  CAGGTTTCCC GACTGGAAAG CGGGCAGTGA GCGCAACGCA ATTAATGTGA GTTAGCTCAC TCATTAGGCA
      GTCCAAAGGG CTGACCTTTC GCCCGTCACT CGCGTTGCGT TAATTACACT CAATCGAGTG AGTAATCCGT

3781  CCCCAGGCTT TACACTTTAT GCTTCCGGCT CGTATGTTGT GTGGAATTGT GAGCGGATAA CAATTTCACA
      GGGGTCCGAA ATGTGAAATA CGAAGGCCGA GCATACAACA CACCTTAACA CTCGCCTATT GTTAAAGTGT

                                        BssHII                             KpnI
                                       ~~~~~~                                ~~
3851  CAGGAAACAG CTATGACCAT GATTACGCCA AGCGCGCAAT TAACCCTCAC TAAAGGGAAC AAAAGCTGGG
      GTCCTTTGTC GATACTGGTA CTAATGCGGT TCGCGCGTTA ATTGGGAGTG ATTTCCCTTG TTTTCGACCC

      KpnI          XhoI
      ~~~~          ~~~~~~
3921  TACCGGGCCC CCCCTCGAGG TCATTCATAT GCTTGAGAAG AGAGTCGGGA TAGTCCAAAA TAAAACAAAG
      ATGGCCCGGG GGGGAGCTCC AGTAAGTATA CGAACTCTTC TCTCAGCCCT ATCAGGTTTT ATTTTGTTTC

3991  GTAAGATTAC CTGGTCAAAA GTGAAAACAT CAGTTAAAAG GTGGTATAAG TAAAATATCG GTAATAAAAG
      CATTCTAATG GACCAGTTTT CACTTTTGTA GTCAATTTTC CACCATATTC ATTTTATAGC CATTATTTTC

4061  GTGGCCCAAA GTGAAATTTA CTCTTTTCTA CTATTATAAA AATTGAGGAT GTTTTGTCGG TACTTTGATA
      CACCGGGTTT CACTTTAAAT GAGAAAAGAT GATAATATTT TTAACTCCTA CAAAACAGCC ATGAAACTAT
```

FIG._45F

```
4131  CGTCATTTTT GTATGAATTG GTTTTTAAGT TTATTCGCGA TTTGGAAATG CATATCTGTA TTTGAGTCGG
      GCAGTAAAAA CATACTTAAC CAAAAATTCA AATAAGCGCT AAACCTTTAC GTATAGACAT AAACTCAGCC

4201  TTTTTAAGTT CGTTGCTTTT GTAAATACAG AGGGATTTGT ATAAGAAATA TCTTTAAAAA ACCCATATGC
      AAAAATTCAA GCAACGAAAA CATTTATGTC TCCCTAAACA TATTCTTTAT AGAAATTTTT TGGGTATACG

                                                  EcoRI
                                                ~~~~~~~~
4271  TAATTTGACA TAATTTTTGA GAAAAATATA TATTCAGGCG AATTCCACAA TGAACAATAA TAAGATTAAA
      ATTAAACTGT ATTAAAAACT CTTTTTATAT ATAAGTCCGC TTAAGGTGTT ACTTGTTATT ATTCTAATTT

4341  ATAGCTTGCC CCCGTTGCAG CGATGGGTAT TTTTTCTAGT AAAATAAAAG ATAAACTTAG ACTCAAAACA
      TATCGAACGG GGGCAACGTC GCTACCCATA AAAAAGATCA TTTTATTTTC TATTTGAATC TGAGTTTTGT

4411  TTTACAAAAA CAACCCCTAA AGTCCTAAAG CCCAAAGTGC TATGCACGAT CCATAGCAAG CCCAGCCCAA
      AAATGTTTTT GTTGGGGATT TCAGGATTTC GGGTTTCACG ATACGTGCTA GGTATCGTTC GGGTCGGGTT
4481  CCCAACCCAA CCCAACCCAC CCCAGTGCAG CCAACTGGCA AATAGTCTCC ACCCCCGGCA CTATCACCGT
      GGGTTGGGTT GGGTTGGGTG GGGTCACGTC GGTTGACCGT TTATCAGAGG TGGGGGCCGT GATAGTGGCA

4551  GAGTTGTCCG CACCACCGCA CGTCTCGCAG CCAAAAAAAA AAAAAGAAAG AAAAAAAAGA AAAAGAAAAA
      CTCAACAGGC GTGGTGGCGT GCAGAGCGTC GGTTTTTTTT TTTTTCTTTC TTTTTTTTCT TTTTCTTTTT

4621  CAGCAGGTGG GTCCGGGTCG TGGGGGCCGG AAAAGCGAGG AGGATCGCGA GCAGCGACGA GGCCCGGCCC
      GTCGTCCACC CAGGCCCAGC ACCCCCGGCC TTTTCGCTCC TCCTAGCGCT CGTCGCTGCT CCGGGCCGGG

4691  TCCCTCCGCT TCCAAAGAAA CGCCCCCCAT CGCCACTATA TACATACCCC CCCCTCTCCT CCCATCCCCC
      AGGGAGGCGA AGGTTTCTTT GCGGGGGGTA GCGGTGATAT ATGTATGGGG GGGGAGAGGA GGGTAGGGGG

4761  CAACCCTACC ACCACCACCA CCACCACCTC CTCCCCCCTC GCTGCCGGAC GACGAGCTCC TCCCCCCTCC
      GTTGGGATGG TGGTGGTGGT GGTGGTGGAG GAGGGGGGAG CGACGGCCTG CTGCTCGAGG AGGGGGGAGG

4831  CCCTCCGCCG CCGCCGGTAA CCACCCCGCC CCTCTCCTCT TTCTTTCTCC GTTTTTTTTT TCGTCTCGGT
      GGGAGGCGGC GGCGGCCATT GGTGGGGCGG GGAGAGGAGA AAGAAAGAGG CAAAAAAAAA AGCAGAGCCA
```

FIG._45G

```
4901  CTCGATCTTT GGCCTTGGTA GTTTGGGTGG GCGAGAGCGG CTTCGTCGCC CAGATCGGTG CGCGGGAGGG
      GAGCTAGAAA CCGGAACCAT CAAACCCACC CGCTCTCGCC GAAGCAGCGG GTCTAGCCAC GCGCCCTCCC

                                                BamHI
                                                ~~~~~~
4971  GCGGGATCTC GCGGCTGGCG TCTCCGGGCG TGAGTCGGCC CGGATCCTCG CGGGGAATGG GGCTCTCGGA
      CGCCCTAGAG CGCCGACCGC AGAGGCCCGC ACTCAGCCGG GCCTAGGAGC GCCCCTTACC CCGAGAGCCT

         BglII
        ~~~~~~
5041  TGTAGATCTT CTTTCTTTCT TCTTTTTGTG GTAGAATTTG AATCCCTCAG CATTGTTCAT CGGTAGTTTT
      ACATCTAGAA GAAAGAAAGA AGAAAAACAC CATCTTAAAC TTAGGGAGTC GTAACAAGTA GCCATCAAAA

5111  TCTTTTCATG ATTTGTGACA AATGCAGCCT CGTGCGGAGC TTTTTTGTAG GTAG
      AGAAAAGTAC TAAACACTGT TTACGTCGGA GCACGCCTCG AAAAAACATC CATC
```

FIG._45H

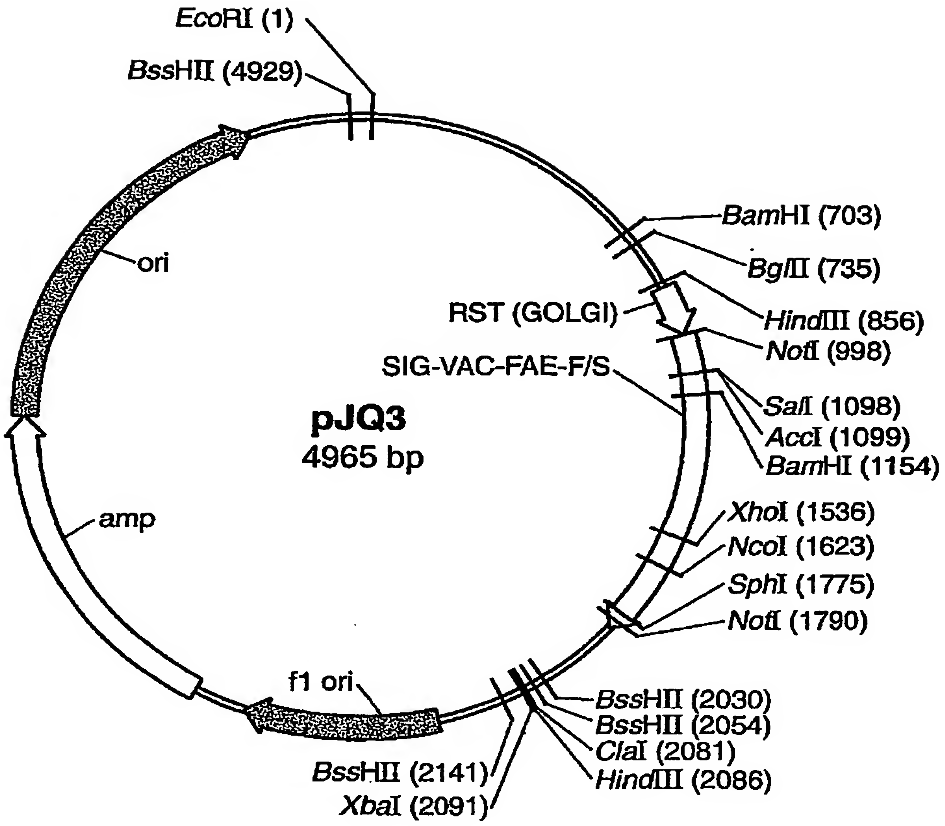

*Eco*RI (1)
*Bss*HII (4929)
*Bam*HI (703)
*Bgl*II (735)
ori
RST (GOLGI)
*Hind*III (856)
*Not*I (998)
SIG-VAC-FAE-F/S
pJQ3
4965 bp
*Sal*I (1098)
*Acc*I (1099)
*Bam*HI (1154)
*Xho*I (1536)
amp
*Nco*I (1623)
*Sph*I (1775)
*Not*I (1790)
f1 ori
*Bss*HII (2030)
*Bss*HII (2054)
*Cla*I (2081)
*Hind*III (2086)
*Bss*HII (2141)
*Xba*I (2091)

FIG._46A

```
      EcoRI
      ~~~~~~
  1   AATTCCACAA TGAACAATAA TAAGATTAAA ATAGCTTGCC CCCGTTGCAG CGATGGGTAT TTTTTCTAGT
      TTAAGGTGTT ACTTGTTATT ATTCTAATTT TATCGAACGG GGGCAACGTC GCTACCCATA AAAAAGATCA

 71   AAAATAAAAG ATAAACTTAG ACTCAAAACA TTTACAAAAA CAACCCCTAA AGTCCTAAAG CCCAAAGTGC
      TTTTATTTTC TATTTGAATC TGAGTTTTGT AAATGTTTTT GTTGGGGATT TCAGGATTTC GGGTTTCACG

141   TATGCACGAT CCATAGCAAG CCCAGCCCAA CCCAACCCAA CCCAACCCAC CCCAGTGCAG CCAACTGGCA
      ATACGTGCTA GGTATCGTTC GGGTCGGGTT GGGTTGGGTT GGGTTGGGTG GGGTCACGTC GGTTGACCGT

211   AATAGTCTCC ACCCCCGGCA CTATCACCGT GAGTTGTCCG CACCACCGCA CGTCTCGCAG CCAAAAAAAA
      TTATCAGAGG TGGGGGCCGT GATAGTGGCA CTCAACAGGC GTGGTGGCGT GCAGAGCGTC GGTTTTTTTT

281   AAAAAGAAAG AAAAAAAAGA AAAAGAAAAA CAGCAGGTGG GTCCGGGTCG TGGGGGCCGG AAAAGCGAGG
      TTTTTCTTTC TTTTTTTTCT TTTTCTTTTT GTCGTCCACC CAGGCCCAGC ACCCCCGGCC TTTTCGCTCC

351   AGGATCGCGA GCAGCGACGA GGCCCGGCCC TCCCTCCGCT TCCAAAGAAA CGCCCCCCAT CGCCACTATA
      TCCTAGCGCT CGTCGCTGCT CCGGGCCGGG AGGGAGGCGA AGGTTTCTTT GCGGGGGGTA GCGGTGATAT

421   TACATACCCC CCCCTCTCCT CCCATCCCCC CAACCCTACC ACCACCACCA CCACCACCTC CTCCCCCCTC
      ATGTATGGGG GGGGAGAGGA GGGTAGGGGG GTTGGGATGG TGGTGGTGGT GGTGGTGGAG GAGGGGGGAG

491   GCTGCCGGAC GACGAGCTCC TCCCCCCTCC CCCTCCGCCG CCGCCGGTAA CCACCCCGCC CCTCTCCTCT
      CGACGGCCTG CTGCTCGAGG AGGGGGGAGG GGGAGGCGGC GGCGGCCATT GGTGGGGCGG GGAGAGGAGA

561   TTCTTTCTCC GTTTTTTTTT TCGTCTCGGT CTCGATCTTT GGCCTTGGTA GTTTGGGTGG GCGAGAGCGG
      AAGAAAGAGG CAAAAAAAAA AGCAGAGCCA GAGCTAGAAA CCGGAACCAT CAAACCCACC CGCTCTCGCC

631   CTTCGTCGCC CAGATCGGTG CGCGGGAGGG GCGGGATCTC GCGGCTGGCG TCTCCGGGCG TGAGTCGGCC
      GAAGCAGCGG GTCTAGCCAC GCGCCCTCCC CGCCCTAGAG CGCCGACCGC AGAGGCCCGC ACTCAGCCGG

        BamHI                             BglII
       ~~~~~~~                           ~~~~~~~
701   CGGATCCTCG CGGGGAATGG GGCTCTCGGA TGTAGATCTT CTTTCTTTCT TCTTTTTGTG GTAGAATTTG
      GCCTAGGAGC GCCCCTTACC CCGAGAGCCT ACATCTAGAA GAAAGAAAGA AGAAAAACAC CATCTTAAAC
```

FIG._46B

```
771   AATCCCTCAG CATTGTTCAT CGGTAGTTTT TCTTTTCATG ATTTGTGACA AATGCAGCCT CGTGCGGAGC
      TTAGGGAGTC GTAACAAGTA GCCATCAAAA AGAAAAGTAC TAAACACTGT TTACGTCGGA GCACGCCTCG

                   HindIII
                    ~~~~~~
841   TTTTTTGTAG GTAGAAGCTT ACCATGATCC ACACCAACCT CAAAAAGAAG TTCTCCCTCT TCATCCTCGT
      AAAAAACATC CATCTTCGAA TGGTACTAGG TGTGGTTGGA GTTTTTCTTC AAGAGGGAGA AGTAGGAGCA

911   CTTCCTCCTC TTCGCCGTGA TCTGCGTGTG GAAGAAGGGC TCCGACTACG AGGCCCTCAC CCTCCAAGCC
      GAAGGAGGAG AAGCGGCACT AGACGCACAC CTTCTTCCCG AGGCTGATGC TCCGGGAGTG GGAGGTTCGG

                        NotI
                     ~~~~~~~~~
981   AAGGAGTTCC AAATGGCGGC CGCCTCCACG CAGGGCATCT CCGAAGACCT CTACAGCCGT TTAGTCGAAA
      TTCCTCAAGG TTTACCGCCG GCGGAGGTGC GTCCCGTAGA GGCTTCTGGA GATGTCGGCA AATCAGCTTT

                                                         SalI
                                                        ~~~~~~~
                                                         AccI
                                                        ~~~~~~~
1051  TGGCCACTAT CTCCCAAGCT GCCTACGCCG ACCTGTGCAA CATTCCGTCG ACTATTATCA AGGGAGAGAA
      ACCGGTGATA GAGGGTTCGA CGGATGCGGC TGGACACGTT GTAAGGCAGC TGATAATAGT TCCCTCTCTT

                                         BamHI
                                         ~~~~~~
1121  AATTTACAAT TCTCAAACTG ACATTAACGG ATGGATCCTC CGCGACGACA GCAGCAAAGA AATAATCACC
      TTAAATGTTA AGAGTTTGAC TGTAATTGCC TACCTAGGAG GCGCTGCTGT CGTCGTTTCT TTATTAGTGG

1191  GTCTTCCGTG GCACTGGTAG TGATACGAAT CTACAACTCG ATACTAACTA CACCCTCACG CCTTTCGACA
      CAGAAGGCAC CGTGACCATC ACTATGCTTA GATGTTGAGC TATGATTGAT GTGGGAGTGC GGAAAGCTGT

1261  CCCTACCACA ATGCAACGGT TGTGAAGTAC ACGGTGGATA TTATATTGGA TGGGTCTCCG TCCAGGACCA
      GGGATGGTGT TACGTTGCCA ACACTTCATG TGCCACCTAT AATATAACCT ACCCAGAGGC AGGTCCTGGT
```

FIG._46C

```
1331   AGTCGAGTCG CTTGTCAAAC AGCAGGTTAG CCAGTATCCG GACTACGCGC TGACCGTGAC CGGCCACKCC
       TCAGCTCAGC GAACAGTTTG TCGTCCAATC GGTCATAGGC CTGATGCGCG ACTGGCACTG GCCGGTGMGG

1401   CTCGGCGCCT CCCTGGCGGC ACTCACTGCC GCCCAGCTGT CTGCGACATA CGACAACATC CGCCTGTACA
       GAGCCGCGGA GGGACCGCCG TGAGTGACGG CGGGTCGACA GACGCTGTAT GCTGTTGTAG GCGGACATGT

                                                                              XhoI
                                                                            ~~~~~~
1471   CCTTCGGCGA ACCGCGCAGC GGCAATCAGG CCTTCGCGTC GTACATGAAC GATGCCTTCC AAGCCTCGAG
       GGAAGCCGCT TGGCGCGTCG CCGTTAGTCC GGAAGCGCAG CATGTACTTG CTACGGAAGG TTCGGAGCTC

1541   CCCAGATACG ACGCAGTATT TCCGGGTCAC TCATGCCAAC GACGGCATCC CAAACCTGCC CCCGGTGGAG
       GGGTCTATGC TGCGTCATAA AGGCCCAGTG AGTACGGTTG CTGCCGTAGG GTTTGGACGG GGGCCACCTC

                   NcoI
                  ~~~~~~
1611   CAGGGGTACG CCCATGGCGG TGTAGAGTAC TGGAGCGTTG ATCCTTACAG CGCCCAGAAC ACATTTGTCT
       GTCCCCATGC GGGTACCGCC ACATCTCATG ACCTCGCAAC TAGGAATGTC GCGGGTCTTG TGTAAACAGA

1681   GCACTGGGGA TGAAGTGCAG TGCTGTGAGG CCCAGGGCGG ACAGGGTGTG AATAATGCGC ACACGACTTA
       CGTGACCCCT ACTTCACGTC ACGACACTCC GGGTCCCGCC TGTCCCACAC TTATTACGCG TGTGCTGAAT

                              SphI                 NotI
                            ~~~~~~~~             ~~~~~~~~~~
1751   TTTTGGGATG ACGAGCGGCG CATGCACCTG GCCGGTCGCG GCCGCGGAAA CCACTGAAGG ATGAGCTGTA
       AAAACCCTAC TGCTCGCCGC GTACGTGGAC CGGCCAGCGC CGGCGCCTTT GGTGACTTCC TACTCGACAT

1821   AAGAAGCAGA TCGTTCAAAC ATTTGGCAAT AAAGTTTCTT AAGATTGAAT CCTGTTGCCG GTCTTGCGAT
       TTCTTCGTCT AGCAAGTTTG TAAACCGTTA TTTCAAAGAA TTCTAACTTA GGACAACGGC CAGAACGCTA

1891   GATTATCATA TAATTTCTGT TGAATTACGT TAAGCATGTA ATAATTAACA TGTAATGCAT GACGTTATTT
       CTAATAGTAT ATTAAAGACA ACTTAATGCA ATTCGTACAT TATTAATTGT ACATTACGTA CTGCAATAAA
```

FIG._46D

```
                                                                               BssHII
                                                                                   ~~
1961    ATGAGATGGG TTTTTATGAT TAGAGTCCCG CAATTATACA TTTAATACGC GATAGAAAAC AAAATATAGC
        TACTCTACCC AAAAATACTA ATCTCAGGGC GTTAATATGT AAATTATGCG CTATCTTTTG TTTTATATCG

                                                                          XbaI
                                                                        ~~~~~~~
        BssHII                  BssHII                       ClaI  HindIII
        ~~~~                    ~~~~~~                      ~~~~~~~~~~~~~
2031    GCGCAAACTA GGATAAATTA TCGCGCGCGG TGTCATCTAT GTTACTAGAT CGATAAGCTT CTAGAGCGGC
        CGCGTTTGAT CCTATTTAAT AGCGCGCGCC ACAGTAGATA CAATGATCTA GCTATTCGAA GATCTCGCCG

                                                  BssHII
                                                  ~~~~~~~
2101    CGGTGGAGCT CCAATTCGCC CTATAGTGAG TCGTATTACG CGCGCTCACT GGCCGTCGTT TTACAACGTC
        GCCACCTCGA GGTTAAGCGG GATATCACTC AGCATAATGC GCGCGAGTGA CCGGCAGCAA AATGTTGCAG

2171    GTGACTGGGA AAACCCTGGC GTTACCCAAC TTAATCGCCT TGCAGCACAT CCCCCTTTCG CCAGCTGGCG
        CACTGACCCT TTTGGGACCG CAATGGGTTG AATTAGCGGA ACGTCGTGTA GGGGGAAAGC GGTCGACCGC

2241    TAATAGCGAA GAGGCCCGCA CCGATCGCCC TTCCCAACAG TTGCGCAGCC TGAATGGCGA ATGGGACGCG
        ATTATCGCTT CTCCGGGCGT GGCTAGCGGG AAGGGTTGTC AACGCGTCGG ACTTACCGCT TACCCTGCGC

2311    CCCTGTAGCG GCGCATTAAG CGCGGCGGGT GTGGTGGTTA CGCGCAGCGT GACCGCTACA CTTGCCAGCG
        GGGACATCGC CGCGTAATTC GCGCCGCCCA CACCACCAAT GCGCGTCGCA CTGGCGATGT GAACGGTCGC

2381    CCCTAGCGCC CGCTCCTTTC GCTTTCTTCC CTTCCTTTCT CGCCACGTTC GCCGGCTTTC CCCGTCAAGC
        GGGATCGCGG GCGAGGAAAG CGAAAGAAGG GAAGGAAAGA GCGGTGCAAG CGGCCGAAAG GGGCAGTTCG

2451    TCTAAATCGG GGGCTCCCTT TAGGGTTCCG ATTTAGTGCT TTACGGCACC TCGACCCCAA AAAACTTGAT
        AGATTTAGCC CCCGAGGGAA ATCCCAAGGC TAAATCACGA AATGCCGTGG AGCTGGGGTT TTTTGAACTA

2521    TAGGGTGATG GTTCACGTAG TGGGCCATCG CCCTGATAGA CGGTTTTTCG CCCTTTGACG TTGGAGTCCA
        ATCCCACTAC CAAGTGCATC ACCCGGTAGC GGGACTATCT GCCAAAAAGC GGGAAACTGC AACCTCAGGT
```

FIG._46E

```
2591  CGTTCTTTAA TAGTGGACTC TTGTTCCAAA CTGGAACAAC ACTCAACCCT ATCTCGGTCT ATTCTTTTGA
      GCAAGAAATT ATCACCTGAG AACAAGGTTT GACCTTGTTG TGAGTTGGGA TAGAGCCAGA TAAGAAAACT

2661  TTTATAAGGG ATTTTGCCGA TTTCGGCCTA TTGGTTAAAA AATGAGCTGA TTTAACAAAA ATTTAACGCG
      AAATATTCCC TAAAACGGCT AAAGCCGGAT AACCAATTTT TTACTCGACT AAATTGTTTT TAAATTGCGC

2731  AATTTTAACA AAATATTAAC GCTTACAATT TAGGTGGCAC TTTTCGGGGA AATGTGCGCG GAACCCCTAT
      TTAAAATTGT TTTATAATTG CGAATGTTAA ATCCACCGTG AAAAGCCCCT TTACACGCGC CTTGGGGATA

2801  TTGTTTATTT TTCTAAATAC ATTCAAATAT GTATCCGCTC ATGAGACAAT AACCCTGATA AATGCTTCAA
      AACAAATAAA AAGATTTATG TAAGTTTATA CATAGGCGAG TACTCTGTTA TTGGGACTAT TTACGAAGTT

2871  TAATATTGAA AAAGGAAGAG TATGAGTATT CAACATTTCC GTGTCGCCCT TATTCCCTTT TTTGCGGCAT
      ATTATAACTT TTTCCTTCTC ATACTCATAA GTTGTAAAGG CACAGCGGGA ATAAGGGAAA AAACGCCGTA

2941  TTTGCCTTCC TGTTTTTGCT CACCCAGAAA CGCTGGTGAA AGTAAAAGAT GCTGAAGATC AGTTGGGTGC
      AAACGGAAGG ACAAAAACGA GTGGGTCTTT GCGACCACTT TCATTTTCTA CGACTTCTAG TCAACCCACG

3011  ACGAGTGGGT TACATCGAAC TGGATCTCAA CAGCGGTAAG ATCCTTGAGA GTTTTCGCCC CGAAGAACGT
      TGCTCACCCA ATGTAGCTTG ACCTAGAGTT GTCGCCATTC TAGGAACTCT CAAAAGCGGG GCTTCTTGCA

3081  TTTCCAATGA TGAGCACTTT TAAAGTTCTG CTATGTGGCG CGGTATTATC CCGTATTGAC GCCGGGCAAG
      AAAGGTTACT ACTCGTGAAA ATTTCAAGAC GATACACCGC GCCATAATAG GGCATAACTG CGGCCCGTTC

3151  AGCAACTCGG TCGCCGCATA CACTATTCTC AGAATGACTT GGTTGAGTAC TCACCAGTCA CAGAAAAGCA
      TCGTTGAGCC AGCGGCGTAT GTGATAAGAG TCTTACTGAA CCAACTCATG AGTGGTCAGT GTCTTTTCGT

3221  TCTTACGGAT GGCATGACAG TAAGAGAATT ATGCAGTGCT GCCATAACCA TGAGTGATAA CACTGCGGCC
      AGAATGCCTA CCGTACTGTC ATTCTCTTAA TACGTCACGA CGGTATTGGT ACTCACTATT GTGACGCCGG

3291  AACTTACTTC TGACAACGAT CGGAGGACCG AAGGAGCTAA CCGCTTTTTT GCACAACATG GGGGATCATG
      TTGAATGAAG ACTGTTGCTA GCCTCCTGGC TTCCTCGATT GGCGAAAAAA CGTGTTGTAC CCCCTAGTAC

3361  TAACTCGCCT TGATCGTTGG GAACCGGAGC TGAATGAAGC CATACCAAAC GACGAGCGTG ACACCACGAT
      ATTGAGCGGA ACTAGCAACC CTTGGCCTCG ACTTACTTCG GTATGGTTTG CTGCTCGCAC TGTGGTGCTA
```

FIG._46F

```
3431  GCCTGTAGCA ATGGCAACAA CGTTGCGCAA ACTATTAACT GGCGAACTAC TTACTCTAGC TTCCCGGCAA
      CGGACATCGT TACCGTTGTT GCAACGCGTT TGATAATTGA CCGCTTGATG AATGAGATCG AAGGGCCGTT

3501  CAATTAATAG ACTGGATGGA GGCGGATAAA GTTGCAGGAC CACTTCTGCG CTCGGCCCTT CCGGCTGGCT
      GTTAATTATC TGACCTACCT CCGCCTATTT CAACGTCCTG GTGAAGACGC GAGCCGGGAA GGCCGACCGA

3571  GGTTTATTGC TGATAAATCT GGAGCCGGTG AGCGTGGGTC TCGCGGTATC ATTGCAGCAC TGGGGCCAGA
      CCAAATAACG ACTATTTAGA CCTCGGCCAC TCGCACCCAG AGCGCCATAG TAACGTCGTG ACCCCGGTCT

3641  TGGTAAGCCC TCCCGTATCG TAGTTATCTA CACGACGGGG AGTCAGGCAA CTATGGATGA ACGAAATAGA
      ACCATTCGGG AGGGCATAGC ATCAATAGAT GTGCTGCCCC TCAGTCCGTT GATACCTACT TGCTTTATCT

3711  CAGATCGCTG AGATAGGTGC CTCACTGATT AAGCATTGGT AACTGTCAGA CCAAGTTTAC TCATATATAC
      GTCTAGCGAC TCTATCCACG GAGTGACTAA TTCGTAACCA TTGACAGTCT GGTTCAAATG AGTATATATG

3781  TTTAGATTGA TTTAAAACTT CATTTTTAAT TTAAAAGGAT CTAGGTGAAG ATCCTTTTTG ATAATCTCAT
      AAATCTAACT AAATTTTGAA GTAAAAATTA AATTTTCCTA GATCCACTTC TAGGAAAAAC TATTAGAGTA

3851  GACCAAAATC CCTTAACGTG AGTTTTCGTT CCACTGAGCG TCAGACCCCG TAGAAAAGAT CAAAGGATCT
      CTGGTTTTAG GGAATTGCAC TCAAAAGCAA GGTGACTCGC AGTCTGGGGC ATCTTTTCTA GTTTCCTAGA

3921  TCTTGAGATC CTTTTTTTCT GCGCGTAATC TGCTGCTTGC AAACAAAAAA ACCACCGCTA CCAGCGGTGG
      AGAACTCTAG GAAAAAAAGA CGCGCATTAG ACGACGAACG TTTGTTTTTT TGGTGGCGAT GGTCGCCACC

3991  TTTGTTTGCC GGATCAAGAG CTACCAACTC TTTTTCCGAA GGTAACTGGC TTCAGCAGAG CGCAGATACC
      AAACAAACGG CCTAGTTCTC GATGGTTGAG AAAAAGGCTT CCATTGACCG AAGTCGTCTC GCGTCTATGG

4061  AAATACTGTC CTTCTAGTGT AGCCGTAGTT AGGCCACCAC TTCAAGAACT CTGTAGCACC GCCTACATAC
      TTTATGACAG GAAGATCACA TCGGCATCAA TCCGGTGGTG AAGTTCTTGA GACATCGTGG CGGATGTATG

4131  CTCGCTCTGC TAATCCTGTT ACCAGTGGCT GCTGCCAGTG GCGATAAGTC GTGTCTTACC GGGTTGGACT
      GAGCGAGACG ATTAGGACAA TGGTCACCGA CGACGGTCAC CGCTATTCAG CACAGAATGG CCCAACCTGA

4201  CAAGACGATA GTTACCGGAT AAGGCGCAGC GGTCGGGCTG AACGGGGGGT TCGTGCACAC AGCCCAGCTT
      GTTCTGCTAT CAATGGCCTA TTCCGCGTCG CCAGCCCGAC TTGCCCCCCA AGCACGTGTG TCGGGTCGAA
```

FIG._46G

```
4271  GGAGCGAACG ACCTACACCG AACTGAGATA CCTACAGCGT GAGCTATGAG AAAGCGCCAC GCTTCCCGAA
      CCTCGCTTGC TGGATGTGGC TTGACTCTAT GGATGTCGCA CTCGATACTC TTTCGCGGTG CGAAGGGCTT

4341  GGGAGAAAGG CGGACAGGTA TCCGGTAAGC GGCAGGGTCG GAACAGGAGA GCGCACGAGG GAGCTTCCAG
      CCCTCTTTCC GCCTGTCCAT AGGCCATTCG CCGTCCCAGC CTTGTCCTCT CGCGTGCTCC CTCGAAGGTC

4411  GGGGAAACGC CTGGTATCTT TATAGTCCTG TCGGGTTTCG CCACCTCTGA CTTGAGCGTC GATTTTTGTG
      CCCCTTTGCG GACCATAGAA ATATCAGGAC AGCCCAAAGC GGTGGAGACT GAACTCGCAG CTAAAAACAC

4481  ATGCTCGTCA GGGGGGCGGA GCCTATGGAA AAACGCCAGC AACGCGGCCT TTTTACGGTT CCTGGCCTTT
      TACGAGCAGT CCCCCCGCCT CGGATACCTT TTTGCGGTCG TTGCGCCGGA AAAATGCCAA GGACCGGAAA

4551  TGCTGGCCTT TTGCTCACAT GTTCTTTCCT GCGTTATCCC CTGATTCTGT GGATAACCGT ATTACCGCCT
      ACGACCGGAA AACGAGTGTA CAAGAAAGGA CGCAATAGGG GACTAAGACA CCTATTGGCA TAATGGCGGA

4621  TTGAGTGAGC TGATACCGCT CGCCGCAGCC GAACGACCGA GCGCAGCGAG TCAGTGAGCG AGGAAGCGGA
      AACTCACTCG ACTATGGCGA GCGGCGTCGG CTTGCTGGCT CGCGTCGCTC AGTCACTCGC TCCTTCGCCT

4691  AGAGCGCCCA ATACGCAAAC CGCCTCTCCC CGCGCGTTGG CCGATTCATT AATGCAGCTG GCACGACAGG
      TCTCGCGGGT TATGCGTTTG GCGGAGAGGG GCGCGCAACC GGCTAAGTAA TTACGTCGAC CGTGCTGTCC

4761  TTTCCCGACT GGAAAGCGGG CAGTGAGCGC AACGCAATTA ATGTGAGTTA GCTCACTCAT TAGGCACCCC
      AAAGGGCTGA CCTTTCGCCC GTCACTCGCG TTGCGTTAAT TACACTCAAT CGAGTGAGTA ATCCGTGGGG

4831  AGGCTTTACA CTTTATGCTT CCGGCTCGTA TGTTGTGTGG AATTGTGAGC GGATAACAAT TTCACACAGG
      TCCGAAATGT GAAATACGAA GGCCGAGCAT ACAACACACC TTAACACTCG CCTATTGTTA AAGTGTGTCC

                                 BssHII                                EcoRI
                                ~~~~~~~~
4901  AAACAGCTAT GACCATGATT ACGCCAAGCG CGCAATTAAC CCTCACTAAA GGGAACAAAA GCTGG
      TTTGTCGATA CTGGTACTAA TGCGGTTCGC GCGTTAATTG GGAGTGATTT CCCTTGTTTT CGACC
```

FIG._46H

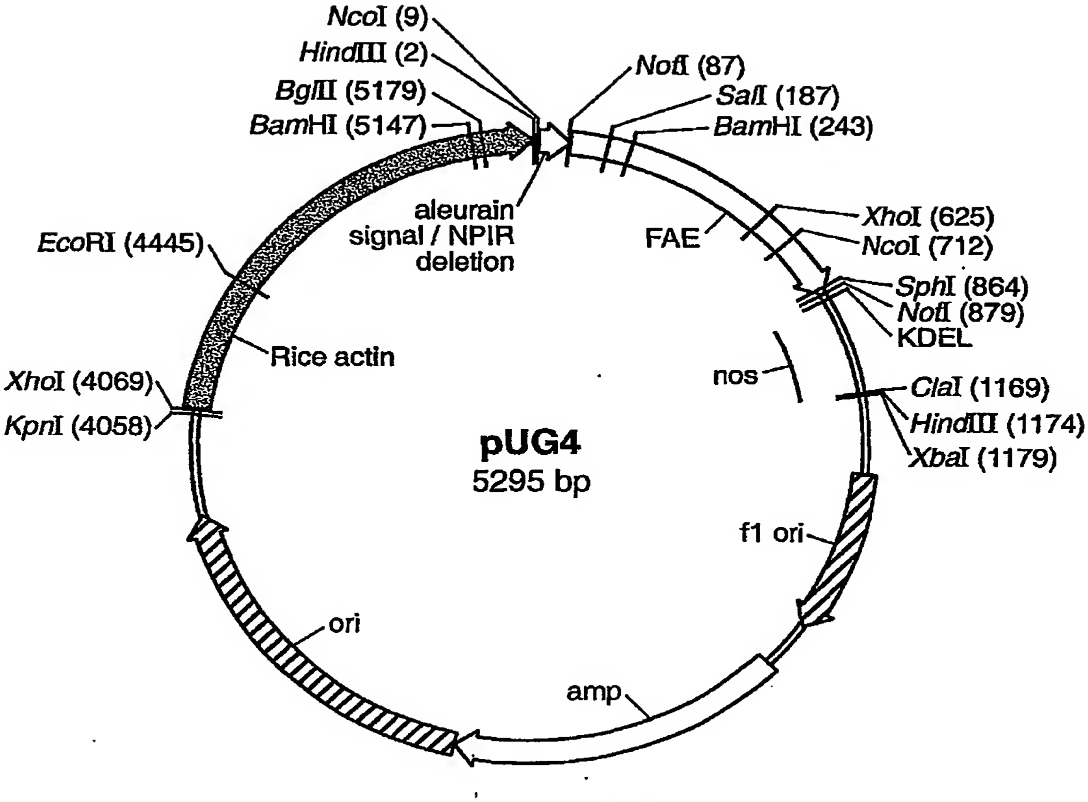

NcoI (9)
HindIII (2)
BglII (5179)
BamHI (5147)
NotI (87)
SalI (187)
BamHI (243)
aleurain signal / NPIR deletion
FAE
XhoI (625)
NcoI (712)
SphI (864)
NotI (879)
KDEL
EcoRI (4445)
Rice actin
nos
ClaI (1169)
HindIII (1174)
XbaI (1179)
XhoI (4069)
KpnI (4058)
pUG4
5295 bp
f1 ori
ori
amp

FIG._47A

```
              NcoI
             ~~~~~~~~
      HindIII
      ~~~~~~
                 M  A  H  A   R  V  L   L  L  A   L  A  V  L   A  T  A   A  V  A   V
    1 AAGCTTACCA TGGCCCACGC CCGCGTCCTC CTCCTGGCGC TCGCCGTGCT GGCCACGGCC GCCGTCGCCG
                       NotI
                     ~~~~~~~~~~
      ·  A  S  S   R  A  A   A  S  T   Q  G  I   S  E  D   L  Y  S   R  L  V  E  M ·
   71 TCGCCTCCTC CCGCGCGGCC GCCTCCACGC AGGGCATCTC CGAAGACCTC TACAGCCGTT TAGTCGAAAT
                                                          SalI
                                                       ~~~~~~~~
      · A  T  I   S  Q  A   A  Y  A  D   L  C  N   I  P  S   T  I  I  K   G  E  K
  141 GGCCACTATC TCCCAAGCTG CCTACGCCGA CCTGTGCAAC ATTCCGTCGA CTATTATCAA GGGAGAGAAA
                                        BamHI
                                        ~~~~~~~
       I  Y  N   S  Q  T  D   I  N  G   W  I  L   R  D  D  S   S  K  E   I  I  T   V
  211 ATTTACAATT CTCAAACTGA CATTAACGGA TGGATCCTCC GCGACGACAG CAGCAAAGAA ATAATCACCG
      ·  F  R  G   T  G  S   D  T  N   L  Q  L   D  T  N   Y  T  L   T  P  F  D  T ·
  281 TCTTCCGTGG CACTGGTAGT GATACGAATC TACAACTCGA TACTAACTAC ACCCTCACGC CTTTCGACAC
      · L  P  Q   C  N  G   C  E  V  H   G  G  Y   Y  I  G   W  V  S  V   Q  D  Q
  351 CCTACCACAA TGCAACGGTT GTGAAGTACA CGGTGGATAT TATATTGGAT GGGTCTCCGT CCAGGACCAA
       V  E  S   L  V  K  Q   Q  V  S   Q  Y  P   D  Y  A  L   T  V  T   G  H  X   L
  421 GTCGAGTCGC TTGTCAAACA GCAGGTTAGC CAGTATCCGG ACTACGCGCT GACCGTGACC GGCCACKCCC
      ·  G  A  S   L  A  A   L  T  A   A  Q  L   S  A  T   Y  D  N   I  R  L  Y  T ·
  491 TCGGCGCCTC CCTGGCGGCA CTCACTGCCG CCCAGCTGTC TGCGACATAC GACAACATCC GCCTGTACAC
                                                                            XhoI
                                                                           ~
      · F  G  E   P  R  S   G  N  Q  A   F  A  S   Y  M  N   D  A  F  Q   A  S  S
  561 CTTCGGCGAA CCGCGCAGCG GCAATCAGGC CTTCGCGTCG TACATGAACG ATGCCTTCCA AGCCTCGAGC
       P  D  T   T  Q  Y  F   R  V  T   H  A  N   D  G  I  P   N  L  P   P  V  E   Q
  631 CCAGATACGA CGCAGTATTT CCGGGTCACT CATGCCAACG ACGGCATCCC AAACCTGCCC CCGGTGGAGC
                  NcoI
                 ~~~~~~~
```

FIG._47B

```
      ·  G  Y  A   H  G  G   V  E  Y   W  S  V  D   P  Y  S   A  Q  N   T  F  V  C·
 701  AGGGGTACGC CCATGGCGGT GTAGAGTACT GGAGCGTTGA TCCTTACAGC GCCCAGAACA CATTTGTCTG
      · T  G  D   E  V  Q   C  C  E  A   Q  G  G   Q  G  V   N  N  A  H   T  T  Y
 771  CACTGGGGAT GAAGTGCAGT GCTGTGAGGC CCAGGGCGGA CAGGGTGTGA ATAATGCGCA CACGACTTAT
                          SphI                 NotI
                         ~~~~~~~~            ~~~~~~~~~~
       F  G  M   T  S  G  A   C  T  W   P  V  A   A  A  E  P   L  K  D   E  L  *
 841  TTTGGGATGA CGAGCGGCGC ATGCACCTGG CCGGTCGCGG CCGCGGAACC ACTGAAGGAT GAGCTGTAAA
 911  GAAGCAGATC GTTCAAACAT TTGGCAATAA AGTTTCTTAA GATTGAATCC TGTTGCCGGT CTTGCGATGA
 981  TTATCATATA ATTTCTGTTG AATTACGTTA AGCATGTAAT AATTAACATG TAATGCATGA CGTTATTTAT
1051  GAGATGGGTT TTTATGATTA GAGTCCCGCA ATTATACATT TAATACGCGA TAGAAAACAA AATATAGCGC
                                                              HindIII
                                                               ~~~~~~~
                                                         ClaI        XbaI
                                                        ~~~~~~~     ~~~~~~~
1121  GCAAACTAGG ATAAATTATC GCGCGCGGTG TCATCTATGT TACTAGATCG ATAAGCTTCT AGAGCGGCCG
1191  GTGGAGCTCC AATTCGCCCT ATAGTGAGTC GTATTACGCG CGCTCACTGG CCGTCGTTTT ACAACGTCGT
1261  GACTGGGAAA ACCCTGGCGT TACCCAACTT AATCGCCTTG CAGCACATCC CCCTTTCGCC AGCTGGCGTA
1331  ATAGCGAAGA GGCCCGCACC GATCGCCCTT CCCAACAGTT GCGCAGCCTG AATGGCGAAT GGGACGCGCC
1401  CTGTAGCGGC GCATTAAGCG CGGCGGGTGT GGTGGTTACG CGCAGCGTGA CCGCTACACT TGCCAGCGCC
1471  CTAGCGCCCG CTCCTTTCGC TTTCTTCCCT TCCTTTCTCG CCACGTTCGC CGGCTTTCCC CGTCAAGCTC
1541  TAAATCGGGG GCTCCCTTTA GGGTTCCGAT TTAGTGCTTT ACGGCACCTC GACCCCAAAA AACTTGATTA
1611  GGGTGATGGT TCACGTAGTG GGCCATCGCC CTGATAGACG GTTTTTCGCC CTTTGACGTT GGAGTCCACG
1681  TTCTTTAATA GTGGACTCTT GTTCCAAACT GGAACAACAC TCAACCCTAT CTCGGTCTAT TCTTTTGATT
1751  TATAAGGGAT TTTGCCGATT TCGGCCTATT GGTTAAAAAA TGAGCTGATT TAACAAAAAT TTAACGCGAA
1821  TTTTAACAAA ATATTAACGC TTACAATTTA GGTGGCACTT TTCGGGGAAA TGTGCGCGGA ACCCCTATTT
1891  GTTTATTTTT CTAAATACAT TCAAATATGT ATCCGCTCAT GAGACAATAA CCCTGATAAA TGCTTCAATA
1961  ATATTGAAAA AGGAAGAGTA TGAGTATTCA ACATTTCCGT GTCGCCCTTA TTCCCTTTTT TGCGGCATTT
2031  TGCCTTCCTG TTTTTGCTCA CCCAGAAACG CTGGTGAAAG TAAAAGATGC TGAAGATCAG TTGGGTGCAC
2101  GAGTGGGTTA CATCGAACTG GATCTCAACA GCGGTAAGAT CCTTGAGAGT TTTCGCCCCG AAGAACGTTT
2171  TCCAATGATG AGCACTTTTA AAGTTCTGCT ATGTGGCGCG GTATTATCCC GTATTGACGC CGGGCAAGAG
2241  CAACTCGGTC GCCGCATACA CTATTCTCAG AATGACTTGG TTGAGTACTC ACCAGTCACA GAAAAGCATC
2311  TTACGGATGG CATGACAGTA AGAGAATTAT GCAGTGCTGC CATAACCATG AGTGATAACA CTGCGGCCAA
2381  CTTACTTCTG ACAACGATCG GAGGACCGAA GGAGCTAACC GCTTTTTTGC ACAACATGGG GGATCATGTA
2451  ACTCGCCTTG ATCGTTGGGA ACCGGAGCTG AATGAAGCCA TACCAAACGA CGAGCGTGAC ACCACGATGC
2521  CTGTAGCAAT GGCAACAACG TTGCGCAAAC TATTAACTGG CGAACTACTT ACTCTAGCTT CCCGGCAACA
```

FIG._47C

```
2591   ATTAATAGAC TGGATGGAGG CGGATAAAGT TGCAGGACCA CTTCTGCGCT CGGCCCTTCC GGCTGGCTGG
2661   TTTATTGCTG ATAAATCTGG AGCCGGTGAG CGTGGGTCTC GCGGTATCAT TGCAGCACTG GGGCCAGATG
2731   GTAAGCCCTC CCGTATCGTA GTTATCTACA CGACGGGGAG TCAGGCAACT ATGGATGAAC GAAATAGACA
2801   GATCGCTGAG ATAGGTGCCT CACTGATTAA GCATTGGTAA CTGTCAGACC AAGTTTACTC ATATATACTT
2871   TAGATTGATT TAAAACTTCA TTTTTAATTT AAAAGGATCT AGGTGAAGAT CCTTTTTGAT AATCTCATGA
2941   CCAAAATCCC TTAACGTGAG TTTTCGTTCC ACTGAGCGTC AGACCCCGTA GAAAAGATCA AAGGATCTTC
3011   TTGAGATCCT TTTTTTCTGC GCGTAATCTG CTGCTTGCAA ACAAAAAAAC CACCGCTACC AGCGGTGGTT
3081   TGTTTGCCGG ATCAAGAGCT ACCAACTCTT TTTCCGAAGG TAACTGGCTT CAGCAGAGCG CAGATACCAA
3151   ATACTGTCCT TCTAGTGTAG CCGTAGTTAG GCCACCACTT CAAGAACTCT GTAGCACCGC CTACATACCT
3221   CGCTCTGCTA ATCCTGTTAC CAGTGGCTGC TGCCAGTGGC GATAAGTCGT GTCTTACCGG GTTGGACTCA
3291   AGACGATAGT TACCGGATAA GGCGCAGCGG TCGGGCTGAA CGGGGGGTTC GTGCACACAG CCCAGCTTGG
3361   AGCGAACGAC CTACACCGAA CTGAGATACC TACAGCGTGA GCTATGAGAA AGCGCCACGC TTCCCGAAGG
3431   GAGAAAGGCG GACAGGTATC CGGTAAGCGG CAGGGTCGGA ACAGGAGAGC GCACGAGGGA GCTTCCAGGG
3501   GGAAACGCCT GGTATCTTTA TAGTCCTGTC GGGTTTCGCC ACCTCTGACT TGAGCGTCGA TTTTTGTGAT
3571   GCTCGTCAGG GGGGCGGAGC CTATGGAAAA ACGCCAGCAA CGCGGCCTTT TTACGGTTCC TGGCCTTTTG
3641   CTGGCCTTTT GCTCACATGT TCTTTCCTGC GTTATCCCCT GATTCTGTGG ATAACCGTAT TACCGCCTTT
3711   GAGTGAGCTG ATACCGCTCG CCGCAGCCGA ACGACCGAGC GCAGCGAGTC AGTGAGCGAG GAAGCGGAAG
3781   AGCGCCCAAT ACGCAAACCG CCTCTCCCCG CGCGTTGGCC GATTCATTAA TGCAGCTGGC ACGACAGGTT
3851   TCCCGACTGG AAAGCGGGCA GTGAGCGCAA CGCAATTAAT GTGAGTTAGC TCACTCATTA GGCACCCCAG
3921   GCTTTACACT TTATGCTTCC GGCTCGTATG TTGTGGAA TTGTGAGCGG ATAACAATTT CACACAGGAA
                                                                            KpnI
                                                                           ~~
3991   ACAGCTATGA CCATGATTAC GCCAAGCGCG CAATTAACCC TCACTAAAGG GAACAAAAGC TGGGTACCGG
              XhoI
             ~~~~~~~~
4061   GCCCCCCCTC GAGGTCATTC ATATGCTTGA GAAGAGAGTC GGGATAGTCC AAAATAAAAC AAAGGTAAGA
4131   TTACCTGGTC AAAAGTGAAA ACATCAGTTA AAAGGTGGTA TAAGTAAAAT ATCGGTAATA AAAGGTGGCC
4201   CAAAGTGAAA TTTACTCTTT TCTACTATTA TAAAAATTGA GGATGTTTTG TCGGTACTTT GATACGTCAT
4271   TTTTGTATGA ATTGGTTTTT AAGTTTATTC GCGATTTGGA AATGCATATC TGTATTTGAG TCGGTTTTTA
4341   AGTTCGTTGC TTTTGTAAAT ACAGAGGGAT TTGTATAAGA AATATCTTTA AAAAACCCAT ATGCTAATTT
                                           EcoRI
                                          ~~~~~~~~
4411   GACATAATTT TTGAGAAAAA TATATATTCA GGCGAATTCC ACAATGAACA ATAATAAGAT TAAAATAGCT
4481   TGCCCCCGTT GCAGCGATGG GTATTTTTTC TAGTAAAATA AAAGATAAAC TTAGACTCAA AACATTTACA
4551   AAAACAACCC CTAAAGTCCT AAAGCCCAAA GTGCTATGCA CGATCCATAG CAAGCCCAGC CCAACCCAAC
4621   CCAACCCAAC CCACCCCAGT GCAGCCAACT GGCAAATAGT CTCCACCCCC GGCACTATCA CCGTGAGTTG
```

FIG._47D

```
4691  TCCGCACCAC CGCACGTCTC GCAGCCAAAA AAAAAAAAAG AAAGAAAAAA AAGAAAAAGA AAAACAGCAG
4761  GTGGGTCCGG GTCGTGGGGG CCGGAAAAGC GAGGAGGATC GCGAGCAGCG ACGAGGCCCG GCCCTCCCTC
4831  CGCTTCCAAA GAAACGCCCC CCATCGCCAC TATATACATA CCCCCCCCTC TCCTCCCATC CCCCCAACCC
4901  TACCACCACC ACCACCACCA CCTCCTCCCC CCTCGCTGCC GGACGACGAG CTCCTCCCCC CTCCCCCTCC
4971  GCCGCCGCCG GTAACCACCC CGCCCCTCTC CTCTTTCTTT CTCCGTTTTT TTTTTCGTCT CGGTCTCGAT
5041  CTTTGGCCTT GGTAGTTTGG GTGGGCGAGA GCGGCTTCGT CGCCCAGATC GGTGCGCGGG AGGGGCGGGA
                                                BamHI                         BglII
                                                ~~~~~~~~
5111  TCTCGCGGCT GGCGTCTCCG GGCGTGAGTC GGCCCGGATC CTCGCGGGGA ATGGGGCTCT CGGATGTAGA
      BglII
      ~~~
5181  TCTTCTTTCT TTCTTCTTTT TGTGGTAGAA TTTGAATCCC TCAGCATTGT TCATCGGTAG TTTTTCTTTT
5251  CATGATTTGT GACAAATGCA GCCTCGTGCG GAGCTTTTTT GTAGC
```

FIG._47E

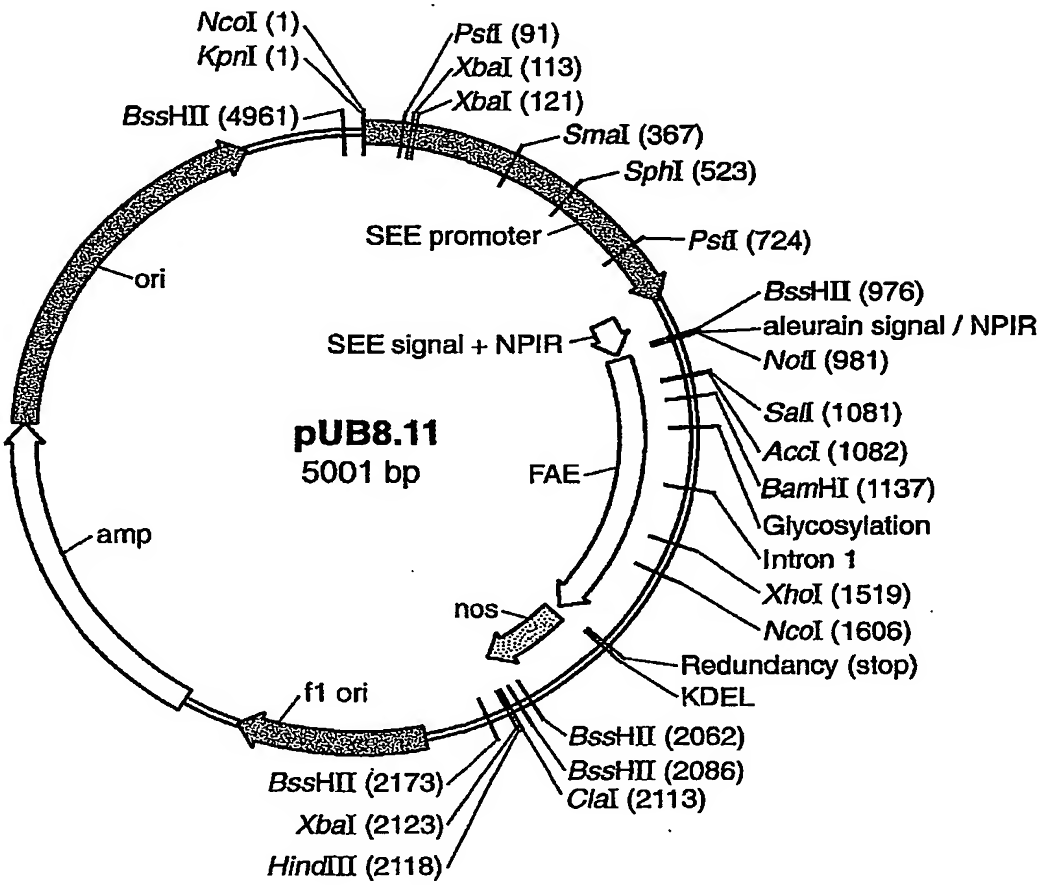

NcoI (1)
KpnI (1)
BssHII (4961)
PstI (91)
XbaI (113)
XbaI (121)
SmaI (367)
SphI (523)
SEE promoter
PstI (724)
ori
BssHII (976)
aleurain signal / NPIR
NotI (981)
SEE signal + NPIR
SalI (1081)
AccI (1082)
BamHI (1137)
Glycosylation
Intron 1
XhoI (1519)
NcoI (1606)
pUB8.11
5001 bp
FAE
amp
nos
Redundancy (stop)
KDEL
f1 ori
BssHII (2062)
BssHII (2086)
ClaI (2113)
BssHII (2173)
XbaI (2123)
HindIII (2118)

FIG._48A

```
      NcoI
      ~~~~~~
      KpnI
      ~
   1  CATGGGCCAG GTATAATTAT GGGATATCTC AAGCAAATAA TCGAAATATC ACCATTGGCT ACAATATCTG
      GTACCCGGTC CATATTAATA CCCTATAGAG TTCGTTTATT AGCTTTATAG TGGTAACCGA TGTTATAGAC

                      PstI                         XbaI     XbaI
                     ~~~~~~~~                     ~~~~~~~ ~~~~~~~~
  71  AGCTCCGAGT TCTGACTGCA GTCTGGATGA CGCGTGTTGT ATCTAGAACT CTAGATAGCA CAGCCACAGC
      TCGAGGCTCA AGACTGACGT CAGACCTACT GCGCACAACA TAGATCTTGA GATCTATCGT GTCGGTGTCG

 141  ACCTACAGGA GTGCGACACT TGTGGACTGT AGTAGTGTTG GAGACGGAGC TCTTTCCTAC CTCCTGACGT
      TGGATGTCCT CACGCTGTGA ACACCTGACA TCATCACAAC CTCTGCCTCG AGAAAGGATG GAGGACTGCA

 211  TGCCGCCGTT GTCCATTCCA ACGGCATCAC TCTCAACCAA TCACGCGCTC CCAACAAAAT ATCGTCCCCC
      ACGGCGGCAA CAGGTAAGGT TGCCGTAGTG AGAGTTGGTT AGTGCGCGAG GGTTGTTTTA TAGCAGGGGG

 281  ATGTCTTGGC GGAGAGAGAG TACATACATG CTGTCGCGCC GTTTTTGTCT GAATCTCGCT TCCACTGGCC
      TACAGAACCG CCTCTCTCTC ATGTATGTAC GACAGCGCGG CAAAAACAGA CTTAGAGCGA AGGTGACCGG

                    SmaI
                   ~~~~~~~
 351  AATCAGCTCA GCTCCCGGGA GCTCACTCAT TCAAGATCCC ATCGTCGTCG TCACCCCTGG CGTCATGGGA
      TTAGTCGAGT CGAGGGCCCT CGAGTGAGTA AGTTCTAGGG TAGCAGCAGC AGTGGGGACC GCAGTACCCT

 421  TGGAAAAGAA CCTCCGTTGC TCGGATGAGT CAGCCATATC CCCGAACAGA GTACTGCAAG ATAACCCAAT
      ACCTTTTCTT GGAGGCAACG AGCCTACTCA GTCGGTATAG GGGCTTGTCT CATGACGTTC TATTGGGTTA

                                     SphI
                                   ~~~~~~~~
 491  TCAGATTCCC CCAATAGAGA AAGTATAGCA TGCTTTCGGG TTTTGTTTGG CTTAATTGAC TTTATTTTTG
      AGTCTAAGGG GGTTATCTCT TTCATATCGT ACGAAAGCCC AAAACAAACC GAATTAACTG AAATAAAAAC

 561  TTGGAGTTGA ATGCTGATTT GTTGTGTAAA ATGCCCAACC ATCTGAATAT CGAGACGGAT AATAGGCTGG
      AACCTCAACT TACGACTAAA CAACACATTT TACGGGTTGG TAGACTTATA GCTCTGCCTA TTATCCGACC
```

FIG._48B

```
 631  CTAATTAATT TATAGCAAGA TTCTGTAGTG CACATCGCAA ATATCTTTCT GGGCATTACA GCTGGAGGCT
      GATTAATTAA ATATCGTTCT AAGACATCAC GTGTAGCGTT TATAGAAAGA CCCGTAATGT CGACCTCCGA

                          PstI
                         ~~~~~~~
 701  TCATCAGCCT GAAACACTCT GCAGAGCCTG AAGCAAGTGG TGAAGCGTGG CGATGAGATG GGTATAAAAC
      AGTAGTCGGA CTTTGTGAGA CGTCTCGGAC TTCGTTCACC ACTTCGCACC GCTACTCTAC CCATATTTTG

 771  CCCCGGCACC GGGACGCGAG CTCCCGCCTA CCAGTACCAT CTCGCCTCGC TCCCCCTGCC GGACGACCCA
      GGGGCCGTGG CCCTGCGCTC GAGGGCGGAT GGTCATGGTA GAGCGGAGCG AGGGGGACGG CCTGCTGGGT

 841  GTAAAATACT GTTGCCCACT CGCCGGCGAG ATGGCCCACG GCCGCATCCT CTTCTTGGCG CTCGCCGTCT
      CATTTTATGA CAACGGGTGA GCGGCCGCTC TACCGGGTGC CGGCGTAGGA GAAGAACCGC GAGCGGCAGA

                                                                            BssHII
                                                                            ~~~~~~
                                                                              NotI
                                                                                ~~
 911  TGGCCACCGC CGCGGTGGCC GCCGCATCNT TGGCGGACTC CAACCCGATC CGGCCCGTCA CCGAGCGCGC
      ACCGGTGGCG GCGCCACCGG CGGCGTAGNA ACCGCCTGAG GTTGGGCTAG GCCGGGCAGT GGCTCGCGCG

      NotI
      ~~~~~~
 981  GGCCGCCTCC ACGCAGGGCA TCTCCGAAGA CCTCTACAGC CGTTTAGTCG AAATGGCCAC TATCTCCCAA
      CCGGCGGAGG TGCGTCCCGT AGAGGCTTCT GGAGATGTCG GCAAATCAGC TTTACCGGTG ATAGAGGGTT

                                       SalI
                                     ~~~~~~~
                                       AccI
                                     ~~~~~~~
1051  GCTGCCTACG CCGACCTGTG CAACATTCCG TCGACTATTA TCAAGGGAGA GAAAATTTAC AATTCTCAAA
      CGACGGATGC GGCTGGACAC GTTGTAAGGC AGCTGATAAT AGTTCCCTCT CTTTTAAATG TTAAGAGTTT
```

FIG._48C

```
                      BamHI
                     ~~~~~~~
1121  CTGACATTAA CGGATGGATC CTCCGCGACG ACAGCAGCAA AGAAATAATC ACCGTCTTCC GTGGCACTGG
      GACTGTAATT GCCTACCTAG GAGGCGCTGC TGTCGTCGTT TCTTTATTAG TGGCAGAAGG CACCGTGACC

1191  TAGTGATACG AATCTACAAC TCGATACTAA CTACACCCTC ACGCCTTTCG ACACCCTACC ACAATGCAAC
      ATCACTATGC TTAGATGTTG AGCTATGATT GATGTGGGAG TGCGGAAAGC TGTGGGATGG TGTTACGTTG

1261  GGTTGTGAAG TACACGGTGG ATATTATATT GGATGGGTCT CCGTCCAGGA CCAAGTCGAG TCGCTTGTCA
      CCAACACTTC ATGTGCCACC TATAATATAA CCTACCCAGA GGCAGGTCCT GGTTCAGCTC AGCGAACAGT

1331  AACAGCAGGT TAGCCAGTAT CCGGACTACG CGCTGACCGT GACCGGCCAC KCCCTCGGCG CCTCCCTGGC
      TTGTCGTCCA ATCGGTCATA GGCCTGATGC GCGACTGGCA CTGGCCGGTG MGGGAGCCGC GGAGGGACCG

1401  GGCACTCACT GCCGCCCAGC TGTCTGCGAC ATACGACAAC ATCCGCCTGT ACACCTTCGG CGAACCGCGC
      CCGTGAGTGA CGGCGGGTCG ACAGACGCTG TATGCTGTTG TAGGCGGACA TGTGGAAGCC GCTTGGCGCG

                                                            XhoI
                                                         ~~~~~~~~
1471  AGCGGCAATC AGGCCTTCGC GTCGTACATG AACGATGCCT TCCAAGCCTC GAGCCCAGAT ACGACGCAGT
      TCGCCGTTAG TCCGGAAGCG CAGCATGTAC TTGCTACGGA AGGTTCGGAG CTCGGGTCTA TGCTGCGTCA

                                                                          NcoI
                                                                        ~~~~~~
1541  ATTTCCGGGT CACTCATGCC AACGACGGCA TCCCAAACCT GCCCCCGGTG GAGCAGGGGT ACGCCCATGG
      TAAAGGCCCA GTGAGTACGG TTGCTGCCGT AGGGTTTGGA CGGGGGCCAC CTCGTCCCCA TGCGGGTACC

1611  CGGTGTAGAG TACTGGAGCG TTGATCCTTA CAGCGCCCAG AACACATTTG TCTGCACTGG GGATGAAGTG
      GCCACATCTC ATGACCTCGC AACTAGGAAT GTCGCGGGTC TTGTGTAAAC AGACGTGACC CCTACTTCAC

1681  CAGTGCTGTG AGGCCCAGGG CGGACAGGGT GTGAATAATG CGCACACGAC TTATTTTGGG ATGACGAGCG
      GTCACGACAC TCCGGGTCCC GCCTGTCCCA CACTTATTAC GCGTGTGCTG AATAAAACCC TACTGCTCGC

1751  GAGCCTGTAC ATGGTGATCA GTCATTTCAG CCTCCCCGAG TGTACCAGGA AAGATGGATG TCCTGGAGAG
      CTCGGACATG TACCACTAGT CAGTAAAGTC GGAGGGGCTC ACATGGTCCT TTCTACCTAC AGGACCTCTC
```

FIG._48D

```
1821  GGGGCCGCGT AACCACTGAA GGATGAGCTG TAAAGAAGCA GATCGTTCAA ACATTTGGCA ATAAAGTTTC
      CCCCGGCGCA TTGGTGACTT CCTACTCGAC ATTTCTTCGT CTAGCAAGTT TGTAAACCGT TATTTCAAAG

1891  TTAAGATTGA ATCCTGTTGC CGGTCTTGCG ATGATTATCA TATAATTTCT GTTGAATTAC GTTAAGCATG
      AATTCTAACT TAGGACAACG GCCAGAACGC TACTAATAGT ATATTAAAGA CAACTTAATG CAATTCGTAC

1961  TAATAATTAA CATGTAATGC ATGACGTTAT TTATGAGATG GGTTTTTATG ATTAGAGTCC CGCAATTATA
      ATTATTAATT GTACATTACG TACTGCAATA AATACTCTAC CCAAAAATAC TAATCTCAGG GCGTTAATAT

                                       BssHII                BssHII
                                       ~~~~~~                ~~~~~~
2031  CATTTAATAC GCGATAGAAA ACAAAATATA GCGCGCAAAC TAGGATAAAT TATCGCGCGC GGTGTCATCT
      GTAAATTATG CGCTATCTTT TGTTTTATAT CGCGCGTTTG ATCCTATTTA ATAGCGCGCG CCACAGTAGA

                             XbaI
                            ~~~~~~
                 ClaI HindIII
                 ~~~~~~~~~~~~~~
2101  ATGTTACTAG ATCGATAAGC TTCTAGAGCG GCCGGTGGAG CTCCAATTCG CCCTATAGTG AGTCGTATTA
      TACAATGATC TAGCTATTCG AAGATCTCGC CGGCCACCTC GAGGTTAAGC GGGATATCAC TCAGCATAAT

       BssHII
      ~~~~~~
2171  CGCGCGCTCA CTGGCCGTCG TTTTACAACG TCGTGACTGG GAAAACCCTG GCGTTACCCA ACTTAATCGC
      GCGCGCGAGT GACCGGCAGC AAAATGTTGC AGCACTGACC CTTTTGGGAC CGCAATGGGT TGAATTAGCG

2241  CTTGCAGCAC ATCCCCCTTT CGCCAGCTGG CGTAATAGCG AAGAGGCCCG CACCGATCGC CCTTCCCAAC
      GAACGTCGTG TAGGGGGAAA GCGGTCGACC GCATTATCGC TTCTCCGGGC GTGGCTAGCG GGAAGGGTTG

2311  AGTTGCGCAG CCTGAATGGC GAATGGGACG CGCCCTGTAG CGGCGCATTA AGCGCGGCGG GTGTGGTGGT
      TCAACGCGTC GGACTTACCG CTTACCCTGC GCGGGACATC GCCGCGTAAT TCGCGCCGCC CACACCACCA

2381  TACGCGCAGC GTGACCGCTA CACTTGCCAG CGCCCTAGCG CCCGCTCCTT TCGCTTTCTT CCCTTCCTTT
      ATGCGCGTCG CACTGGCGAT GTGAACGGTC GCGGGATCGC GGGCGAGGAA AGCGAAAGAA GGGAAGGAAA
```

FIG._48E

```
2451  CTCGCCACGT TCGCCGGCTT TCCCCGTCAA GCTCTAAATC GGGGGCTCCC TTTAGGGTTC CGATTTAGTG
      GAGCGGTGCA AGCGGCCGAA AGGGGCAGTT CGAGATTTAG CCCCCGAGGG AAATCCCAAG GCTAAATCAC

2521  CTTTACGGCA CCTCGACCCC AAAAAACTTG ATTAGGGTGA TGGTTCACGT AGTGGGCCAT CGCCCTGATA
      GAAATGCCGT GGAGCTGGGG TTTTTTGAAC TAATCCCACT ACCAAGTGCA TCACCCGGTA GCGGGACTAT

2591  GACGGTTTTT CGCCCTTTGA CGTTGGAGTC CACGTTCTTT AATAGTGGAC TCTTGTTCCA AACTGGAACA
      CTGCCAAAAA GCGGGAAACT GCAACCTCAG GTGCAAGAAA TTATCACCTG AGAACAAGGT TTGACCTTGT

2661  ACACTCAACC CTATCTCGGT CTATTCTTTT GATTTATAAG GGATTTTGCC GATTTCGGCC TATTGGTTAA
      TGTGAGTTGG GATAGAGCCA GATAAGAAAA CTAAATATTC CCTAAAACGG CTAAAGCCGG ATAACCAATT

2731  AAAATGAGCT GATTTAACAA AAATTTAACG CGAATTTTAA CAAAATATTA ACGCTTACAA TTTAGGTGGC
      TTTTACTCGA CTAAATTGTT TTTAAATTGC GCTTAAAATT GTTTTATAAT TGCGAATGTT AAATCCACCG

2801  ACTTTTCGGG GAAATGTGCG CGGAACCCCT ATTTGTTTAT TTTTCTAAAT ACATTCAAAT ATGTATCCGC
      TGAAAAGCCC CTTTACACGC GCCTTGGGGA TAAACAAATA AAAAGATTTA TGTAAGTTTA TACATAGGCG

2871  TCATGAGACA ATAACCCTGA TAAATGCTTC AATAATATTG AAAAAGGAAG AGTATGAGTA TTCAACATTT
      AGTACTCTGT TATTGGGACT ATTTACGAAG TTATTATAAC TTTTTCCTTC TCATACTCAT AAGTTGTAAA

2941  CCGTGTCGCC CTTATTCCCT TTTTTGCGGC ATTTTGCCTT CCTGTTTTTG CTCACCCAGA AACGCTGGTG
      GGCACAGCGG GAATAAGGGA AAAAACGCCG TAAAACGGAA GGACAAAAAC GAGTGGGTCT TTGCGACCAC

3011  AAAGTAAAAG ATGCTGAAGA TCAGTTGGGT GCACGAGTGG GTTACATCGA ACTGGATCTC AACAGCGGTA
      TTTCATTTTC TACGACTTCT AGTCAACCCA CGTGCTCACC CAATGTAGCT TGACCTAGAG TTGTCGCCAT

3081  AGATCCTTGA GAGTTTTCGC CCCGAAGAAC GTTTTCCAAT GATGAGCACT TTTAAAGTTC TGCTATGTGG
      TCTAGGAACT CTCAAAAGCG GGGCTTCTTG CAAAAGGTTA CTACTCGTGA AAATTTCAAG ACGATACACC

3151  CGCGGTATTA TCCCGTATTG ACGCCGGGCA AGAGCAACTC GGTCGCCGCA TACACTATTC TCAGAATGAC
      GCGCCATAAT AGGGCATAAC TGCGGCCCGT TCTCGTTGAG CCAGCGGCGT ATGTGATAAG AGTCTTACTG

3221  TTGGTTGAGT ACTCACCAGT CACAGAAAAG CATCTTACGG ATGGCATGAC AGTAAGAGAA TTATGCAGTG
      AACCAACTCA TGAGTGGTCA GTGTCTTTTC GTAGAATGCC TACCGTACTG TCATTCTCTT AATACGTCAC
```

FIG._48F

```
3291  CTGCCATAAC CATGAGTGAT AACACTGCGG CCAACTTACT TCTGACAACG ATCGGAGGAC CGAAGGAGCT
      GACGGTATTG GTACTCACTA TTGTGACGCC GGTTGAATGA AGACTGTTGC TAGCCTCCTG GCTTCCTCGA

3361  AACCGCTTTT TTGCACAACA TGGGGGATCA TGTAACTCGC CTTGATCGTT GGGAACCGGA GCTGAATGAA
      TTGGCGAAAA AACGTGTTGT ACCCCCTAGT ACATTGAGCG GAACTAGCAA CCCTTGGCCT CGACTTACTT

3431  GCCATACCAA ACGACGAGCG TGACACCACG ATGCCTGTAG CAATGGCAAC AACGTTGCGC AAACTATTAA
      CGGTATGGTT TGCTGCTCGC ACTGTGGTGC TACGGACATC GTTACCGTTG TTGCAACGCG TTTGATAATT

3501  CTGGCGAACT ACTTACTCTA GCTTCCCGGC AACAATTAAT AGACTGGATG GAGGCGGATA AAGTTGCAGG
      GACCGCTTGA TGAATGAGAT CGAAGGGCCG TTGTTAATTA TCTGACCTAC CTCCGCCTAT TTCAACGTCC

3571  ACCACTTCTG CGCTCGGCCC TTCCGGCTGG CTGGTTTATT GCTGATAAAT CTGGAGCCGG TGAGCGTGGG
      TGGTGAAGAC GCGAGCCGGG AAGGCCGACC GACCAAATAA CGACTATTTA GACCTCGGCC ACTCGCACCC

3641  TCTCGCGGTA TCATTGCAGC ACTGGGGCCA GATGGTAAGC CCTCCCGTAT CGTAGTTATC TACACGACGG
      AGAGCGCCAT AGTAACGTCG TGACCCCGGT CTACCATTCG GGAGGGCATA GCATCAATAG ATGTGCTGCC

3711  GGAGTCAGGC AACTATGGAT GAACGAAATA GACAGATCGC TGAGATAGGT GCCTCACTGA TTAAGCATTG
      CCTCAGTCCG TTGATACCTA CTTGCTTTAT CTGTCTAGCG ACTCTATCCA CGGAGTGACT AATTCGTAAC

3781  GTAACTGTCA GACCAAGTTT ACTCATATAT ACTTTAGATT GATTTAAAAC TTCATTTTTA ATTTAAAAGG
      CATTGACAGT CTGGTTCAAA TGAGTATATA TGAAATCTAA CTAAATTTTG AAGTAAAAAT TAAATTTTCC

3851  ATCTAGGTGA AGATCCTTTT TGATAATCTC ATGACCAAAA TCCCTTAACG TGAGTTTTCG TTCCACTGAG
      TAGATCCACT TCTAGGAAAA ACTATTAGAG TACTGGTTTT AGGGAATTGC ACTCAAAAGC AAGGTGACTC

3921  CGTCAGACCC CGTAGAAAAG ATCAAAGGAT CTTCTTGAGA TCCTTTTTTT CTGCGCGTAA TCTGCTGCTT
      GCAGTCTGGG GCATCTTTTC TAGTTTCCTA GAAGAACTCT AGGAAAAAAA GACGCGCATT AGACGACGAA

3991  GCAAACAAAA AAACCACCGC TACCAGCGGT GGTTTGTTTG CCGGATCAAG AGCTACCAAC TCTTTTTCCG
      CGTTTGTTTT TTTGGTGGCG ATGGTCGCCA CCAAACAAAC GGCCTAGTTC TCGATGGTTG AGAAAAAGGC

4061  AAGGTAACTG GCTTCAGCAG AGCGCAGATA CCAAATACTG TCCTTCTAGT GTAGCCGTAG TTAGGCCACC
      TTCCATTGAC CGAAGTCGTC TCGCGTCTAT GGTTTATGAC AGGAAGATCA CATCGGCATC AATCCGGTGG
```

FIG._48G

```
4131  ACTTCAAGAA CTCTGTAGCA CCGCCTACAT ACCTCGCTCT GCTAATCCTG TTACCAGTGG CTGCTGCCAG
      TGAAGTTCTT GAGACATCGT GGCGGATGTA TGGAGCGAGA CGATTAGGAC AATGGTCACC GACGACGGTC

4201  TGGCGATAAG TCGTGTCTTA CCGGGTTGGA CTCAAGACGA TAGTTACCGG ATAAGGCGCA GCGGTCGGGC
      ACCGCTATTC AGCACAGAAT GGCCCAACCT GAGTTCTGCT ATCAATGGCC TATTCCGCGT CGCCAGCCCG

4271  TGAACGGGGG GTTCGTGCAC ACAGCCCAGC TTGGAGCGAA CGACCTACAC CGAACTGAGA TACCTACAGC
      ACTTGCCCCC CAAGCACGTG TGTCGGGTCG AACCTCGCTT GCTGGATGTG GCTTGACTCT ATGGATGTCG

4341  GTGAGCTATG AGAAAGCGCC ACGCTTCCCG AAGGGAGAAA GGCGGACAGG TATCCGGTAA GCGGCAGGGT
      CACTCGATAC TCTTTCGCGG TGCGAAGGGC TTCCCTCTTT CCGCCTGTCC ATAGGCCATT CGCCGTCCCA

4411  CGGAACAGGA GAGCGCACGA GGGAGCTTCC AGGGGGAAAC GCCTGGTATC TTTATAGTCC TGTCGGGTTT
      GCCTTGTCCT CTCGCGTGCT CCCTCGAAGG TCCCCCTTTG CGGACCATAG AAATATCAGG ACAGCCCAAA

4481  CGCCACCTCT GACTTGAGCG TCGATTTTTG TGATGCTCGT CAGGGGGGCG GAGCCTATGG AAAAACGCCA
      GCGGTGGAGA CTGAACTCGC AGCTAAAAAC ACTACGAGCA GTCCCCCCGC CTCGGATACC TTTTTGCGGT

4551  GCAACGCGGC CTTTTTACGG TTCCTGGCCT TTTGCTGGCC TTTTGCTCAC ATGTTCTTTC CTGCGTTATC
      CGTTGCGCCG GAAAAATGCC AAGGACCGGA AAACGACCGG AAAACGAGTG TACAAGAAAG GACGCAATAG

4621  CCCTGATTCT GTGGATAACC GTATTACCGC CTTTGAGTGA GCTGATACCG CTCGCCGCAG CCGAACGACC
      GGGACTAAGA CACCTATTGG CATAATGGCG GAAACTCACT CGACTATGGC GAGCGGCGTC GGCTTGCTGG

4691  GAGCGCAGCG AGTCAGTGAG CGAGGAAGCG GAAGAGCGCC CAATACGCAA ACCGCCTCTC CCCGCGCGTT
      CTCGCGTCGC TCAGTCACTC GCTCCTTCGC CTTCTCGCGG GTTATGCGTT TGGCGGAGAG GGGCGCGCAA

4761  GGCCGATTCA TTAATGCAGC TGGCACGACA GGTTTCCCGA CTGGAAAGCG GGCAGTGAGC GCAACGCAAT
      CCGGCTAAGT AATTACGTCG ACCGTGCTGT CCAAAGGGCT GACCTTTCGC CCGTCACTCG CGTTGCGTTA

4831  TAATGTGAGT TAGCTCACTC ATTAGGCACC CCAGGCTTTA CACTTTATGC TTCCGGCTCG TATGTTGTGT
      ATTACACTCA ATCGAGTGAG TAATCCGTGG GGTCCGAAAT GTGAAATACG AAGGCCGAGC ATACAACACA
```

FIG._48H

```
                                                                                    BssHII
                                                                                   ~~~~~~~
4901  GGAATTGTGA GCGGATAACA ATTTCACACA GGAAACAGCT ATGACCATGA TTACGCCAAG CGCGCAATTA
      CCTTAACACT CGCCTATTGT TAAAGTGTGT CCTTTGTCGA TACTGGTACT AATGCGGTTC GCGCGTTAAT

                                  NcoI

                                  KpnI
                                 ~~~~~
4971  ACCCTCACTA AAGGGAACAA AAGCTGGGTA C
      TGGGAGTGAT TTCCCTTGTT TTCGACCCAT G
```

FIG._48I

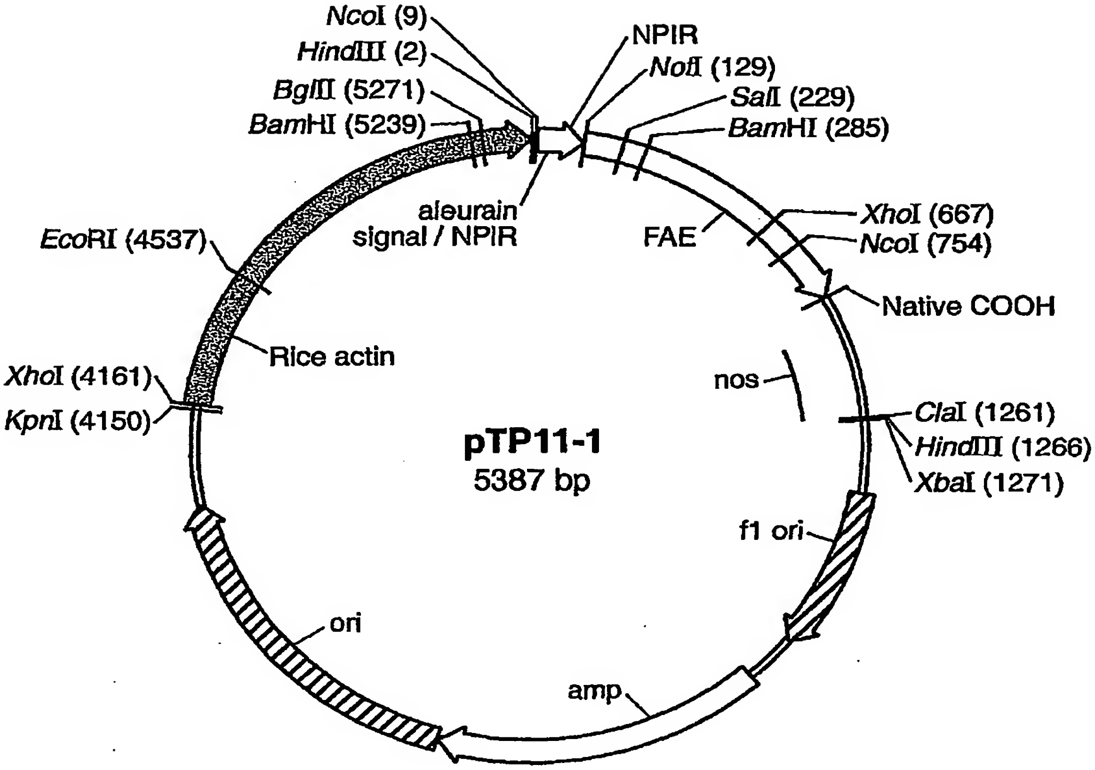

NcoI (9)
HindIII (2)
BglII (5271)
BamHI (5239)
NPIR
NotI (129)
SalI (229)
BamHI (285)
aleurain signal / NPIR
FAE
XhoI (667)
NcoI (754)
Native COOH
EcoRI (4537)
Rice actin
nos
XhoI (4161)
KpnI (4150)
pTP11-1
5387 bp
ClaI (1261)
HindIII (1266)
XbaI (1271)
f1 ori
ori
amp

FIG._49A

```
                  NcoI
                ~~~~~~~~
     HindIII
     ~~~~~~
                M  A  H  A   R  V  L   L  L  A  L   A  V  L   A  T  A   A  V  A  V
   1 AAGCTTACCA TGGCCCACGC CCGCGTCCTC CTCCTGGCGC TCGCCGTGCT GGCCACGGCC GCCGTCGCCG
                                                                    NotI
                                                                  ~~~~~~~~~
     •  A  S  S   S  S  F   A  D  S  N   P  I  R   P  V  T   D  R  A  A   A  S  T •
  71 TCGCCTCCTC CTCCTCCTTC GCCGACTCCA ACCCGATCCG GCCCGTCACC GACCGCGCGG CCGCCTCCAC
     • Q  G  I   S  E  D  L   Y  S  R   L  V  E   M  A  T  I   S  Q  A   A  Y  A
 141 GCAGGGCATC TCCGAAGACC TCTACAGCCG TTTAGTCGAA ATGGCCACTA TCTCCCAAGC TGCCTACGCC
                   SalI
                 ~~~~~~~~
                   AccI
                 ~~~~~~~~
      D  L  C  N   I  P  S   T  I  I   K  G  E  K   I  Y  N   S  Q  T   D  I  N  G
 211 GACCTGTGCA ACATTCCGTC GACTATTATC AAGGGAGAGA AAATTTACAA TTCTCAAACT GACATTAACG
       BamHI
       ~~~~~~
     •  W  I  L   R  D  D   S  S  K  E   I  I  T   V  F  R   G  T  G  S   D  T  N •
 281 GATGGATCCT CCGCGACGAC AGCAGCAAAG AAATAATCAC CGTCTTCCGT GGCACTGGTA GTGATACGAA
     • L  Q  L   D  T  N  Y   T  L  T   P  F  D   T  L  P  Q   C  N  G   C  E  V
 351 TCTACAACTC GATACTAACT ACACCCTCAC GCCTTTCGAC ACCCTACCAC AATGCAACGG TTGTGAAGTA
      H  G  G  Y   Y  I  G   W  V  S   V  Q  D  Q   V  E  S   L  V  K   Q  Q  V  S
 421 CACGGTGGAT ATTATATTGG ATGGGTCTCC GTCCAGGACC AAGTCGAGTC GCTTGTCAAA CAGCAGGTTA
     •  Q  Y  P   D  Y  A   L  T  V  T   G  H  X   L  G  A   S  L  A  A   L  T  A •
 491 GCCAGTATCC GGACTACGCG CTGACCGTGA CCGGCCACKC CCTCGGCGCC TCCCTGGCGG CACTCACTGC
     • A  Q  L   S  A  T  Y   D  N  I   R  L  Y   T  F  G  E   P  R  S   G  N  Q
 561 CGCCCAGCTG TCTGCGACAT ACGACAACAT CCGCCTGTAC ACCTTCGGCG AACCGCGCAG CGGCAATCAG
                                               XhoI
                                             ~~~~~~~~
      A  F  A  S   Y  M  N   D  A  F   Q  A  S  S   P  D  T   T  Q  Y   F  R  V  T
 631 GCCTTCGCGT CGTACATGAA CGATGCCTTC CAAGCCTCGA GCCCAGATAC GACGCAGTAT TTCCGGGTCA
```

FIG._49B

```
                                                                 NcoI
                                                               ~~~~~~~
      ·  H  A  N   D  G  I   P  N  L   P  P  V  E   Q  G  Y   A  H  G   G  V  E  Y ·
 701  CTCATGCCAA CGACGGCATC CCAAACCTGC CCCCGGTGGA GCAGGGGTAC GCCCATGGCG GTGTAGAGTA
       ·W  S  V   D  P  Y   S  A  Q  N   T  F  V   C  T  G   D  E  V  Q   C  C  E
 771  CTGGAGCGTT GATCCTTACA GCGCCCAGAA CACATTTGTC TGCACTGGGG ATGAAGTGCA GTGCTGTGAG
       A  Q  G   G  Q  G  V   N  N  A   H  T  T   Y  F  G  M   T  S  G   A  C  T   W
 841  GCCCAGGGCG GACAGGGTGT GAATAATGCG CACACGACTT ATTTTGGGAT GACGAGCGGA GCCTGTACAT
      ·  *
 911  GGTGATCAGT CATTTCAGCC TCCCCGAGTG TACCAGGAAA GATGGATGTC CTGGAGAGGG GGCCGCGTAA
 981  CCACTGAAGG ATGAGCTGTA AAGAAGCAGA TCGTTCAAAC ATTTGGCAAT AAAGTTTCTT AAGATTGAAT
1051  CCTGTTGCCG GTCTTGCGAT GATTATCATA TAATTTCTGT TGAATTACGT TAAGCATGTA ATAATTAACA
1121  TGTAATGCAT GACGTTATTT ATGAGATGGG TTTTTATGAT TAGAGTCCCG CAATTATACA TTTAATACGC
                                                                              ClaI
1191  GATAGAAAAC AAAATATAGC GCGCAAACTA GGATAAATTA TCGCGCGCGG TGTCATCTAT GTTACTAGAT
          HindIII
          ~~~~~~~~
      ClaI       XbaI
      ~~~~      ~~~~~~~~
1261  CGATAAGCTT CTAGAGCGGC CGGTGGAGCT CCAATTCGCC CTATAGTGAG TCGTATTACG CGCGCTCACT
1331  GGCCGTCGTT TTACAACGTC GTGACTGGGA AAACCCTGGC GTTACCCAAC TTAATCGCCT TGCAGCACAT
1401  CCCCCTTTCG CCAGCTGGCG TAATAGCGAA GAGGCCCGCA CCGATCGCCC TTCCCAACAG TTGCGCAGCC
1471  TGAATGGCGA ATGGGACGCG CCCTGTAGCG GCGCATTAAG CGCGGCGGGT GTGGTGGTTA CGCGCAGCGT
1541  GACCGCTACA CTTGCCAGCG CCCTAGCGCC CGCTCCTTTC GCTTTCTTCC CTTCCTTTCT CGCCACGTTC
1611  GCCGGCTTTC CCCGTCAAGC TCTAAATCGG GGGCTCCCTT TAGGGTTCCG ATTTAGTGCT TTACGGCACC
1681  TCGACCCCAA AAAACTTGAT TAGGGTGATG GTTCACGTAG TGGGCCATCG CCCTGATAGA CGGTTTTTCG
1751  CCCTTTGACG TTGGAGTCCA CGTTCTTTAA TAGTGGACTC TTGTTCCAAA CTGGAACAAC ACTCAACCCT
1821  ATCTCGGTCT ATTCTTTTGA TTTATAAGGG ATTTTGCCGA TTTCGGCCTA TTGGTTAAAA AATGAGCTGA
1891  TTTAACAAAA ATTTAACGCG AATTTTAACA AAATATTAAC GCTTACAATT TAGGTGGCAC TTTTCGGGGA
1961  AATGTGCGCG GAACCCCTAT TTGTTTATTT TTCTAAATAC ATTCAAATAT GTATCCGCTC ATGAGACAAT
2031  AACCCTGATA AATGCTTCAA TAATATTGAA AAAGGAAGAG TATGAGTATT CAACATTTCC GTGTCGCCCT
2101  TATTCCCTTT TTTGCGGCAT TTTGCCTTCC TGTTTTTGCT CACCCAGAAA CGCTGGTGAA AGTAAAAGAT
2171  GCTGAAGATC AGTTGGGTGC ACGAGTGGGT TACATCGAAC TGGATCTCAA CAGCGGTAAG ATCCTTGAGA
2241  GTTTTCGCCC CGAAGAACGT TTTCCAATGA TGAGCACTTT TAAAGTTCTG CTATGTGGCG CGGTATTATC
2311  CCGTATTGAC GCCGGGCAAG AGCAACTCGG TCGCCGCATA CACTATTCTC AGAATGACTT GGTTGAGTAC
2381  TCACCAGTCA CAGAAAAGCA TCTTACGGAT GGCATGACAG TAAGAGAATT ATGCAGTGCT GCCATAACCA
```

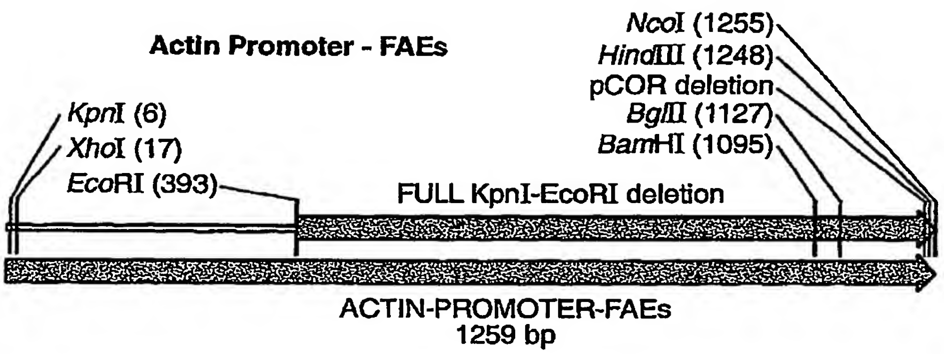
FIG._49C

```
2451  TGAGTGATAA CACTGCGGCC AACTTACTTC TGACAACGAT CGGAGGACCG AAGGAGCTAA CCGCTTTTTT
2521  GCACAACATG GGGGATCATG TAACTCGCCT TGATCGTTGG GAACCGGAGC TGAATGAAGC CATACCAAAC
2591  GACGAGCGTG ACACCACGAT GCCTGTAGCA ATGGCAACAA CGTTGCGCAA ACTATTAACT GGCGAACTAC
2661  TTACTCTAGC TTCCCGGCAA CAATTAATAG ACTGGATGGA GGCGGATAAA GTTGCAGGAC CACTTCTGCG
2731  CTCGGCCCTT CCGGCTGGCT GGTTTATTGC TGATAAATCT GGAGCCGGTG AGCGTGGGTC TCGCGGTATC
2801  ATTGCAGCAC TGGGGCCAGA TGGTAAGCCC TCCCGTATCG TAGTTATCTA CACGACGGGG AGTCAGGCAA
2871  CTATGGATGA ACGAAATAGA CAGATCGCTG AGATAGGTGC CTCACTGATT AAGCATTGGT AACTGTCAGA
2941  CCAAGTTTAC TCATATATAC TTTAGATTGA TTTAAAACTT CATTTTTAAT TTAAAAGGAT CTAGGTGAAG
3011  ATCCTTTTTG ATAATCTCAT GACCAAAATC CCTTAACGTG AGTTTTCGTT CCACTGAGCG TCAGACCCCG
3081  TAGAAAAGAT CAAAGGATCT TCTTGAGATC CTTTTTTTCT GCGCGTAATC TGCTGCTTGC AAACAAAAAA
3151  ACCACCGCTA CCAGCGGTGG TTTGTTTGCC GGATCAAGAG CTACCAACTC TTTTTCCGAA GGTAACTGGC
3221  TTCAGCAGAG CGCAGATACC AAATACTGTC CTTCTAGTGT AGCCGTAGTT AGGCCACCAC TTCAAGAACT
3291  CTGTAGCACC GCCTACATAC CTCGCTCTGC TAATCCTGTT ACCAGTGGCT GCTGCCAGTG GCGATAAGTC
3361  GTGTCTTACC GGGTTGGACT CAAGACGATA GTTACCGGAT AAGGCGCAGC GGTCGGGCTG AACGGGGGGT
3431  TCGTGCACAC AGCCCAGCTT GGAGCGAACG ACCTACACCG AACTGAGATA CCTACAGCGT GAGCTATGAG
3501  AAAGCGCCAC GCTTCCCGAA GGGAGAAAGG CGGACAGGTA TCCGGTAAGC GGCAGGGTCG GAACAGGAGA
3571  GCGCACGAGG GAGCTTCCAG GGGGAAACGC CTGGTATCTT TATAGTCCTG TCGGGTTTCG CCACCTCTGA
3641  CTTGAGCGTC GATTTTTGTG ATGCTCGTCA GGGGGGCGGA GCCTATGGAA AAACGCCAGC AACGCGGCCT
3711  TTTTACGGTT CCTGGCCTTT TGCTGGCCTT TTGCTCACAT GTTCTTTCCT GCGTTATCCC CTGATTCTGT
3781  GGATAACCGT ATTACCGCCT TTGAGTGAGC TGATACCGCT CGCCGCAGCC GAACGACCGA GCGCAGCGAG
3851  TCAGTGAGCG AGGAAGCGGA AGAGCGCCCA ATACGCAAAC CGCCTCTCCC CGCGCGTTGG CCGATTCATT
3921  AATGCAGCTG GCACGACAGG TTTCCCGACT GGAAAGCGGG CAGTGAGCGC AACGCAATTA ATGTGAGTTA
3991  GCTCACTCAT TAGGCACCCC AGGCTTTACA CTTTATGCTT CCGGCTCGTA TGTTGTGTGG AATTGTGAGC
4061  GGATAACAAT TTCACACAGG AAACAGCTAT GACCATGATT ACGCCAAGCG CGCAATTAAC CCTCACTAAA
                     KpnI              XhoI
                    ~~~~~~~~         ~~~~~~~

4131  GGGAACAAAA GCTGGGTACC GGGCCCCCCC TCGAGGTCAT TCATATGCTT GAGAAGAGAG TCGGGATAGT
4201  CCAAAATAAA ACAAAGGTAA GATTACCTGG TCAAAAGTGA AAACATCAGT TAAAAGGTGG TATAAGTAAA
4271  ATATCGGTAA TAAAAGGTGG CCCAAAGTGA AATTTACTCT TTTCTACTAT TATAAAAATT GAGGATGTTT
4341  TGTCGGTACT TTGATACGTC ATTTTTGTAT GAATTGGTTT TTAAGTTTAT TCGCGATTTG GAAATGCATA
4411  TCTGTATTTG AGTCGGTTTT TAAGTTCGTT GCTTTTGTAA ATACAGAGGG ATTTGTATAA GAAATATCTT
                                                                 EcoRI
                                                                 ~~~~~~~

4481  TAAAAAACCC ATATGCTAAT TTGACATAAT TTTTGAGAAA AATATATATT CAGGCGAATT CCACAATGAA
4551  CAATAATAAG ATTAAAATAG CTTGCCCCCG TTGCAGCGAT GGGTATTTTT TCTAGTAAAA TAAAAGATAA
4621  ACTTAGACTC AAAACATTTA CAAAAACAAC CCCTAAAGTC CTAAAGCCCA AAGTGCTATG CACGATCCAT
```

FIG._49D

```
4691  AGCAAGCCCA GCCCAACCCA ACCCAACCCA ACCCACCCCA GTGCAGCCAA CTGGCAAATA GTCTCCACCC
4761  CCGGCACTAT CACCGTGAGT TGTCCGCACC ACCGCACGTC TCGCAGCCAA AAAAAAAAAA AGAAAGAAAA
4831  AAAAGAAAAA GAAAAACAGC AGGTGGGTCC GGGTCGTGGG GGCCGGAAAA GCGAGGAGGA TCGCGAGCAG
4901  CGACGAGGCC CGGCCCTCCC TCCGCTTCCA AAGAAACGCC CCCCATCGCC ACTATATACA TACCCCCCCC
4971  TCTCCTCCCA TCCCCCCAAC CCTACCACCA CCACCACCAC CACCTCCTCC CCCCTCGCTG CCGGACGACG
5041  AGCTCCTCCC CCCTCCCCCT CCGCCGCCGC CGGTAACCAC CCCGCCCCTC TCCTCTTTCT TTCTCCGTTT
5111  TTTTTTTCGT CTCGGTCTCG ATCTTTGGCC TTGGTAGTTT GGGTGGGCGA GAGCGGCTTC GTCGCCCAGA
                                                                   BamHI
                                                                   ~~~~~~
5181  TCGGTGCGCG GGAGGGGCGG GATCTCGCGG CTGGCGTCTC CGGGCGTGAG TCGGCCCGGA TCCTCGCGGG
                         BglII
                         ~~~~~~~
5251  GAATGGGGCT CTCGGATGTA GATCTTCTTT CTTTCTTCTT TTTGTGGTAG AATTTGAATC CCTCAGCATT
5321  GTTCATCGGT AGTTTTTCTT TTCATGATTT GTGACAAATG CAGCCTCGTG CGGAGCTTTT TTGTAGC
```

FIG._49E



```
        KpnI             XhoI
        ~~~~~~           ~~~~~~~

  1  GGTACCGGGC CCCCCCTCGA GGTCATTCAT ATGCTTGAGA AGAGAGTCGG GATAGTCCAA AATAAAACAA
     CCATGGCCCG GGGGGGAGCT CCAGTAAGTA TACGAACTCT TCTCTCAGCC CTATCAGGTT TTATTTTGTT

 71  AGGTAAGATT ACCTGGTCAA AAGTGAAAAC ATCAGTTAAA AGGTGGTATA AGTAAAATAT CGGTAATAAA
     TCCATTCTAA TGGACCAGTT TTCACTTTTG TAGTCAATTT TCCACCATAT TCATTTTATA GCCATTATTT

141  AGGTGGCCCA AAGTGAAATT TACTCTTTTC TACTATTATA AAAATTGAGG ATGTTTTGTC GGTACTTTGA
     TCCACCGGGT TTCACTTTAA ATGAGAAAAG ATGATAATAT TTTTAACTCC TACAAAACAG CCATGAAACT

211  TACGTCATTT TTGTATGAAT TGGTTTTTAA GTTTATTCGC GATTTGGAAA TGCATATCTG TATTTGAGTC
     ATGCAGTAAA AACATACTTA ACCAAAAATT CAAATAAGCG CTAAACCTTT ACGTATAGAC ATAAACTCAG

281  GGTTTTTAAG TTCGTTGCTT TTGTAAATAC AGAGGGATTT GTATAAGAAA TATCTTTAAA AAACCCATAT
     CCAAAAATTC AAGCAACGAA AACATTTATG TCTCCCTAAA CATATTCTTT ATAGAAATTT TTTGGGTATA

                                                  EcoRI
                                                 ~~~~~~

351  GCTAATTTGA CATAATTTTT GAGAAAAATA TATATTCAGG CGAATTCCAC AATGAACAAT AATAAGATTA
     CGATTAAACT GTATTAAAAA CTCTTTTTAT ATATAAGTCC GCTTAAGGTG TTACTTGTTA TTATTCTAAT

421  AAATAGCTTG CCCCCGTTGC AGCGATGGGT ATTTTTTCTA GTAAAATAAA AGATAAACTT AGACTCAAAA
     TTTATCGAAC GGGGGCAACG TCGCTACCCA TAAAAAAGAT CATTTTATTT TCTATTTGAA TCTGAGTTTT

491  CATTTACAAA AACAACCCCT AAAGTCCTAA AGCCCAAAGT GCTATGCACG ATCCATAGCA AGCCCAGCCC
     GTAAATGTTT TTGTTGGGGA TTTCAGGATT TCGGGTTTCA CGATACGTGC TAGGTATCGT TCGGGTCGGG

561  AACCCAACCC AACCCAACCC ACCCCAGTGC AGCCAACTGG CAAATAGTCT CCACCCCCGG CACTATCACC
     TTGGGTTGGG TTGGGTTGGG TGGGGTCACG TCGGTTGACC GTTTATCAGA GGTGGGGGCC GTGATAGTGG

631  GTGAGTTGTC CGCACCACCG CACGTCTCGC AGCCAAAAAA AAAAAAAGAA AGAAAAAAAA GAAAAAGAAA
     CACTCAACAG GCGTGGTGGC GTGCAGAGCG TCGGTTTTTT TTTTTTTCTT TCTTTTTTTT CTTTTTCTTT

701  AACAGCAGGT GGGTCCGGGT CGTGGGGGCC GGAAAAGCGA GGAGGATCGC GAGCAGCGAC GAGGCCCGGC
     TTGTCGTCCA CCCAGGCCCA GCACCCCCGG CCTTTTCGCT CCTCCTAGCG CTCGTCGCTG CTCCGGGCCG
```

FIG._50A

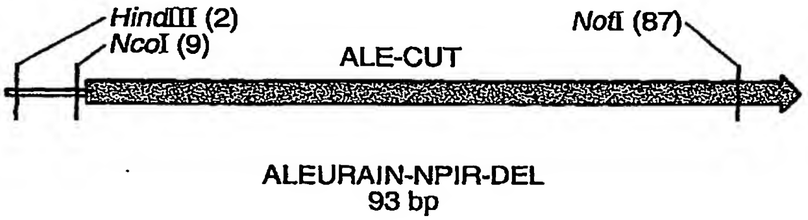
SUBSTITUTE SHEET (RULE 26)

```
 771  CCTCCCTCCG CTTCCAAAGA AACGCCCCCC ATCGCCACTA TATACATACC CCCCCCTCTC CTCCCATCCC
      GGAGGGAGGC GAAGGTTTCT TTGCGGGGGG TAGCGGTGAT ATATGTATGG GGGGGGAGAG GAGGGTAGGG

 841  CCCAACCCTA CCACCACCAC CACCACCACC TCCTCCCCCC TCGCTGCCGG ACGACGAGCT CCTCCCCCCT
      GGGTTGGGAT GGTGGTGGTG GTGGTGGTGG AGGAGGGGGG AGCGACGGCC TGCTGCTCGA GGAGGGGGGA

 911  CCCCCTCCGC CGCCGCCGGT AACCACCCCG CCCCTCTCCT CTTTCTTTCT CCGTTTTTTT TTTCGTCTCG
      GGGGGAGGCG GCGGCGGCCA TTGGTGGGGC GGGGAGAGGA GAAAGAAAGA GGCAAAAAAA AAAGCAGAGC

 981  GTCTCGATCT TTGGCCTTGG TAGTTTGGGT GGGCGAGAGC GGCTTCGTCG CCCAGATCGG TGCGCGGGAG
      CAGAGCTAGA AACCGGAACC ATCAAACCCA CCCGCTCTCG CCGAAGCAGC GGGTCTAGCC ACGCGCCCTC

                                                     BamHI
                                                     ~~~~~~
1051  GGGCGGGATC TCGCGGCTGG CGTCTCCGGG CGTGAGTCGG CCCGGATCCT CGCGGGGAAT GGGGCTCTCG
      CCCGCCCTAG AGCGCCGACC GCAGAGGCCC GCACTCAGCC GGGCCTAGGA GCGCCCCTTA CCCCGAGAGC

           BglII
           ~~~~~~~
1121  GATGTAGATC TTCTTTCTTT CTTCTTTTTG TGGTAGAATT TGAATCCCTC AGCATTGTTC ATCGGTAGTT
      CTACATCTAG AAGAAAGAAA GAAGAAAAAC ACCATCTTAA ACTTAGGGAG TCGTAACAAG TAGCCATCAA

                                                                   HindIII NcoI
                                                                   ~~~~~~~ ~~~~~~
1191  TTTCTTTTCA TGATTTGTGA CAAATGCAGC CTCGTGCGGA GCTTTTTTGT AGGTAGAAGC TTACCATGG
      AAAGAAAAGT ACTAAACACT GTTTACGTCG GAGCACGCCT CGAAAAAACA TCCATCTTCG AATGGTACC
```

KpnI-EcoRI - deletion underlined and restored NCO site in bold in vectors pJQ4.9, pJQ3.2 and pJO6.3.

FIG._50B

ALEURAIN_deleted NPIR (Apoplast) Structure and Sequence



```
+1              M  A  H  A  R  V  L  L  L  A  L  A  V  L  A  T  A  A  V  A
   HindIII NcoI
   ~~~~~~ ~~~~~~~

1  AAGCTTACCA TGGCCCACGC CCGCGTCCTC CTCCTGGCGC TCGCCGTGCT GGCCACGGCC GCCGTCGCCG
   TTCGAATGGT ACCGGGTGCG GGCGCAGGAG GAGGACCGCG AGCGGCACGA CCGGTGCCGG CGGCAGCGGC

+1 V  A  S  S  R  A  A
                NotI
              ~~~~~~~~~

71 TCGCCTCCTC CCGCGCGGCC GCC
   AGCGGAGGAG GGCGCGCCGG CGG
```

FIG._51

SEE1 (Senescence enhanced) PROMOTER sequence

```
   1    CATGGGCCAG GTATAATTAT GGGATATCTC AAGCAAATAA TCGAAATATC ACCATTGGCT ACAATATCTG
                         PstI                         XbaI    XbaI
                        ~~~~~~~                      ~~~~~~~ ~~~~~~
  71    AGCTCCGAGT TCTGACTGCA GTCTGGATGA CGCGTGTTGT ATCTAGAACT CTAGATAGCA CAGCCACAGC
 141    ACCTACAGGA GTGCGACACT TGTGGACTGT AGTAGTGTTG GAGACGGAGC TCTTTCCTAC CTCCTGACGT
 211    TGCCGCCGTT GTCCATTCCA ACGGCATCAC TCTCAACCAA TCACGCGCTC CCAACAAAAT ATCGTCCCCC
 281    ATGTCTTGGC GGAGAGAGAG TACATACATG CTGTCGCGCC GTTTTTGTCT GAATCTCGCT TCCACTGGCC
                       SmaI
                      ~~~~~~~
 351    AATCAGCTCA GCTCCCGGGA GCTCACTCAT TCAAGATCCC ATCGTCGTCG TCACCCCTGG CGTCATGGGA
 421    TGGAAAAGAA CCTCCGTTGC TCGGATGAGT CAGCCATATC CCCGAACAGA GTACTGCAAG ATAACCCAAT
                                      SphI
                                     ~~~~~~~
 491    TCAGATTCCC CCAATAGAGA AAGTATAGCA TGCTTTCGGG TTTTGTTTGG CTTAATTGAC TTTATTTTTG
 561    TTGGAGTTGA ATGCTGATTT GTTGTGTAAA ATGCCCAACC ATCTGAATAT CGAGACGGAT AATAGGCTGG
 631    CTAATTAATT TATAGCAAGA TTCTGTAGTG CACATCGCAA ATATCTTTCT GGGCATTACA GCTGGAGGCT
                            PstI
                           ~~~~~~~
 701    TCATCAGCCT GAAACACTCT GCAGAGCCTG AAGCAAGTGG TGAAGCGTGG CGATGAGATG GGTATAAAAC
 771    CCCCGGCACC GGGACGCGAG CTCCCGCCTA CCAGTACCAT CTCGCCTCGC TCCCCCTGCC GGACGACCCA
 841    GTAAAATACT GTTGCCCACT CGCCGGCGAG ATG
```

FIG._52

SEE1 (Senescence enhanced) PROMOTER plus vacuolar aleurain SIGNAL/NPIR sequence

```
   1    CATGGGCCAG GTATAATTAT GGGATATCTC AAGCAAATAA TCGAAATATC ACCATTGGCT ACAATATCTG
                         PstI                         XbaI    XbaI
                        ~~~~~~~                      ~~~~~~~ ~~~~~~
  71    AGCTCCGAGT TCTGACTGCA GTCTGGATGA CGCGTGTTGT ATCTAGAACT CTAGATAGCA CAGCCACAGC
 141    ACCTACAGGA GTGCGACACT TGTGGACTGT AGTAGTGTTG GAGACGGAGC TCTTTCCTAC CTCCTGACGT
 211    TGCCGCCGTT GTCCATTCCA ACGGCATCAC TCTCAACCAA TCACGCGCTC CCAACAAAAT ATCGTCCCCC
 281    ATGTCTTGGC GGAGAGAGAG TACATACATG CTGTCGCGCC GTTTTTGTCT GAATCTCGCT TCCACTGGCC
                       SmaI
                      ~~~~~~~
 351    AATCAGCTCA GCTCCCGGGA GCTCACTCAT TCAAGATCCC ATCGTCGTCG TCACCCCTGG CGTCATGGGA
 421    TGGAAAAGAA CCTCCGTTGC TCGGATGAGT CAGCCATATC CCCGAACAGA GTACTGCAAG ATAACCCAAT
                                      SphI
                                     ~~~~~~~
 491    TCAGATTCCC CCAATAGAGA AAGTATAGCA TGCTTTCGGG TTTTGTTTGG CTTAATTGAC TTTATTTTTG
 561    TTGGAGTTGA ATGCTGATTT GTTGTGTAAA ATGCCCAACC ATCTGAATAT CGAGACGGAT AATAGGCTGG
 631    CTAATTAATT TATAGCAAGA TTCTGTAGTG CACATCGCAA ATATCTTTCT GGGCATTACA GCTGGAGGCT
                            PstI
                           ~~~~~~~
 701    TCATCAGCCT GAAACACTCT GCAGAGCCTG AAGCAAGTGG TGAAGCGTGG CGATGAGATG GGTATAAAAC
 771    CCCCGGCACC GGGACGCGAG CTCCCGCCTA CCAGTACCAT CTCGCCTCGC TCCCCCTGCC GGACGACCCA
                                          M  A  H  G   R  I  L   F  L  A   L  A  V  L
 841    GTAAAATACT GTTGCCCACT CGCCGGCGAG ATGGCCCACG GCCGCATCCT CTTCTTGGCG CTCGCCGTCT
                                                                              BssHII
                                                                                  NotI
        ·  A  T  A   A  V  A   A  A  S  L   A  D  S   N  P  I   R  P  V  T   E  R  A ·
 911    TGGCCACCGC CGCGGTGGCC GCCGCATCNT TGGCGGACTC CAACCCGATC CGGCCCGTCA CCGAGCGCGC
         NotI
        ~~~~~~
        · A  A
 981    GGCCGCC
```

FIG._53

INTERNATIONAL SEARCH REPORT

Intern Application No
PCT/US 01/43588

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/82

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

WPI Data, EPO-Internal, PAJ, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 6 143 543 A (MICHELSEN BIRGIT ET AL) 7 November 2000 (2000-11-07) see the whole document	1-3, 34-36,74
Y		4-33, 37-73
Y	DE VRIES R P ET AL: "The faeA genes from Aspergillus niger and Aspergillus tubingensis encode ferulic acid esterases involved in degradation of complex cell wall polysaccharides." APPLIED AND ENVIRONMENTAL MICROBIOLOGY, vol. 63, no. 12, December 1997 (1997-12), pages 4638-4644, XP002203731 ISSN: 0099-2240 see the whole document	4-33, 37-73

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☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Date of the actual completion of the international search
27 June 2002

Date of mailing of the international search report
12/07/2002

Name and mailing address of the ISA
European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer
Grosskopf, R

Form PCT/ISA/210 (second sheet) (July 1992)

Inten l Application No
PCT/US 01/43588

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	GARCIA-CONESA MARIA-TERESA ET AL: "A cinnamoyl esterase from Aspergillus niger can break plant cell wall cross-links without release of free diferulic acids." EUROPEAN JOURNAL OF BIOCHEMISTRY, vol. 266, no. 2, December 1999 (1999-12), pages 644-652, XP002203732 ISSN: 0014-2956	

INTERNATIONAL SEARCH REPORT

Information on patent family members

Interr Application No
PCT/US 01/43588

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 6143543 A	07-11-2000	GB 2301103 A ,B	27-11-1996